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CONTENTS

SEC. C.—BOTANICAL SCIENCES

Page

Flax Studies. III. A Refractometric Method for the Estimation of Iodine Value of Raw Linseed Oil—*F. H. Lehberg and W. F. Geddes* - - - - - - - - - - - - - 349

Inheritance of Quality and Quantity of Oil in Flax in Relation to Other Plant Characters—*W. G. McGregor* - - - - - 362

Chemical Weed Killers. II. Factors Affecting Estimation of Toxicity of Leaf Sprays—*W. H. Cook* - - - - - - - 380

The Protective Layers of the Apple—*Hugh P. Bell* - - - - 391

SEC. D.—ZOOLOGICAL SCIENCES

A Preliminary Account of the Unionidae of Ontario—*A. La Rocque and J. Oughton* - - - - - - - - - - - - 147

Some Observations on the Overwintering of Certain Helminth Parasites of Sheep in Canada—*Henry J. Griffiths* - - - 156

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FLAX STUDIES

III. A REFRACTOMETRIC METHOD FOR THE ESTIMATION OF IODINE VALUE OF RAW LINSEED OIL[1]

BY F. H. LEHBERG[2] AND W. F. GEDDES[3]

Abstract

A rapid refractometric method for the estimation of the iodine value of freshly prepared linseed oil is described. This involves expression of the oil at laboratory temperature from finely ground flaxseed, determining the refractive index at 25° C. and converting to Wijs iodine value by means of the regression equation expressing the relation between these variables. In a study of cold pressed linseed oils prepared from 339 samples of sound Canadian flaxseed varying in Wijs iodine value from 153 to 202 units, a correlation of 0.980 between refractive index and Wijs iodine value was found which permits the estimation of iodine value with a standard error of prediction of 2.1 units.

Ethyl ether extracts obtained by the usual extraction procedure for determining oil content are not recommended, owing to differences in the characteristics of the oil and a lower degree of association between refractive index and iodine value.

Since the refractive index of linseed oil and its relation to iodine value are influenced by free fatty acids, oxidation and polymerization, the method is not applicable to commercially prepared oils nor to flaxseed which has heated or become musty.

The method is considered sufficiently accurate for surveys of the quality of flaxseed produced in different districts and of new varieties and hybrids submitted by plant breeders. It should also prove of considerable utility to linseed crushers for securing a measure of the intrinsic drying value of their raw material.

Introduction

The commercial value of flaxseed depends primarily on the quantity and quality (drying properties) of the linseed oil it is capable of yielding. The drying properties depend upon the extent and rate of oxygen absorption, both of which are associated mainly with the total degree of unsaturation. While the rate of oxidation of linolenic, linoleic and oleic acids decreases in the order named, and hence is influenced in part by the relative proportions of these acids present as glycerides, the total degree of unsaturation, as measured by iodine value, has been found to give a valuable and fairly reliable index of the drying quality of linseed oils, and is used extensively in commercial practice.

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[2] *Junior Chemist, Grain Research Laboratory, Board of Grain Commissioners, Winnipeg, Manitoba.*

[3] *Chemist-in-charge, Grain Research Laboratory, Board of Grain Commissioners, Winnipeg, Manitoba.*

The quantity and quality of the oil in flaxseed vary over wide ranges, depending upon variety and the climatic conditions under which it is produced; for example, surveys conducted by this laboratory on commercial flaxseed marketed in Western Canada during 1934, 1935 and 1936, reveal a range in oil content from 35.2% to 45.2%, on a dry matter basis, and in iodine value from 168.5 to 201.7, the lowest values being obtained in 1936 owing to the unusually dry weather and high temperatures which prevailed during the growing season. These variations represent wide differences in commercial value and it has been shown by Coleman and Fellows (6), Birchard (5), Geddes (8, 9) and Geddes and Lehberg (11), that the physical factors used officially for the grading of flaxseed in the United States and Canada fail to differentiate flaxseed satisfactorily on a quality basis. However, the conventional chemical methods for determining oil content and quality are not suitable for routine grading purposes, and convenient rapid and accurate methods are required to place the grading of flaxseed on a scientific basis. Such methods would also be of great utility in the development of new varieties, in zoning the producing areas and in the linseed oil industry.

These considerations have stimulated research leading to the development of rapid methods which would be sufficiently accurate for these purposes. In the second paper of this series (12), we reported on an improved refractometric method for the estimation of oil content and gave the results of some preliminary studies on the relation between refractive index and iodine value. It is the purpose of the present paper to present the experimental work that led to the development of a rapid refractometric method for the estimation of iodine value.

A review of the literature reveals that, while it has been commonly recognized that a relation exists between certain physical and chemical constants of fatty oils and fats, it is only recently that any attempt has been made to utilize the correlation between refractive index and iodine value as a basis for a rapid analytical procedure. Lewkowitsch (15) classified the physical and chemical constants of a large number of oils, fats and waxes but failed to find a definite relation between refractive index and iodine value; this is contrary to the findings of Arnold (2) and Backer (4) who showed that iodine value, saponification number and refractive index were correlated. Niegemann and Kayser (17) reported that the iodine value of linseed oil may be approximated from a knowledge of the refractive index if the source of the oil is known. Lund (16), Pickering and Cowlishaw (19) and others have derived equations expressing the mathematical relations between refractive index and the chemical constants of fatty oils and fats. In the instance of freshly prepared linseed, soya bean, cottonseed and peanut oils, which have similar saponification numbers and low free fatty acid content, the latter workers found the simple equation:—$n_D^{40} = 1.4515 + 0.00\,0117$ (iodine value) to hold. They studied the effect on refractive index of variations in free fatty

acid content, molecular weight, oxidized and hydroxylated acids and developed the equation—

$$n_D^{40} = 1.4643 - 0.000058 \text{ (saponification value)} - \frac{0.0096 \text{ acid value}}{\text{saponification number}} + 0.000117 \text{ (iodine value)}$$

—which applied to linseed, soya bean, cottonseed, peanut, rapeseed, palm nut and cocoanut oils. It was pointed out that this formula only held for freshly prepared oils from sound material, as the refractive index increases with age owing to the fact that the decrease with increasing acidity is smaller than the increase caused by oxidation and polymerization.

From theoretical considerations, Wolff (21) confirmed Lund's experimentally developed relations and derived the expression: $n = 1 + d\,(0.5557 - 0.00022s + 0.000035I)$ where n = refractive index, d = density, s = acid number, and I = iodine value, the formula giving "n" values within 0.0003 units of those found experimentally. This formula holds good for the glycerides of the aliphatic series, but not for the hydroxy or cyclic acids.

Sudborough, Watson and Athawale (20) investigated the refractive index and iodine number of hydrogenated oils and found the relation between these two variables to be independent of the hydrogenation time and type of catalyst and to be virtually identical for cottonseed, linseed, peanut, mohua, sesame and sardine oils. The equation $n_D^{60} = 1.4468 + 1.03 \times 10^{-4}$ (iodine value) $+ 7.3 \times 10^{-3}$ (iodine value)^2 quite accurately represented the relations found.

Lathrap (14), reporting on perilla oil, stressed the close relation between refractive index and iodine value.

Pickard (18) stated that the refractive index of linseed oil varies with the source of the oil and, on pure untreated raw oils, increases with specific gravity and iodine value.

In the second paper of this series (12), we reported a correlation of 0.647 between the refractive index and iodine value of linseed oils extracted by diethyl ether. It was mentioned that the refractive index of ethyl ether extracts appeared to be sensitive to variations in the temperature at which the extracts were prepared and that these did not appear to be accompanied by corresponding changes in iodine value. It was suggested that a closer association between these variables might be obtained in expressed oils.

Recently, Zeleny and Coleman (22, 23) and Hopper and Nesbitt (13) have also described refractometric methods for estimating iodine value, based on the correlation between refractive index and iodine number. Zeleny and Coleman cited the correlation coefficient of 0.647 between the refractive index and iodine number of oils extracted with diethyl ether, reported in the second paper of our series, but overlooked, apparently, the report by Geddes (10) that he had found a correlation of 0.95 between the refractive index and iodine number of cold pressed oil.

Experimental

As the work of Lund (16) and Pickering and Cowlishaw (19) has shown that the relation between refractive index and iodine value is influenced by free fatty acids, oxidation and polymerization, only sound samples were employed in this study. They represented commercial carlots of various grades of Western Canadian flaxseed marketed in 1934 and 1935, and several varieties and hybrids submitted by Canadian plant breeders for quality tests. In view of the fact that the authors (12) had previously secured a relatively low correlation between the refractive index and iodine value of linseed oils extracted by diethyl ether, a comparative study of these constants and their interrelations was made on ether-extracted and cold pressed linseed oils from 110 samples of flaxseed. The samples were ground to a fine pulp in a Hobart model 6-burr mill (equipped with stationary burr No. 4317, No. 2 R.N. Sta. MCH No. 6 Ex P.G. and rotary burr No. 4318, No. 2 P.H. Rot. MCH No. 6 Ex P.G.) with a setting of from 5.0 to 5.5.

The ethyl ether extracts were prepared by extracting a 10-gm. sample of the flax pulp, which had been dried overnight *in vacuo* at 98 to 100° C., with anhydrous alcohol-free and peroxide-free ethyl ether for 16 hr. in a Soxhlet extractor using Whatman double-thickness thimbles and a siphoning rate of one per minute. In order to remove traces of starch, the extracts were filtered through a sintered glass filter and transferred directly to a 125 cc. Erlenmeyer flask, the extraction flask being washed three times with fresh solvent. The excess ether was distilled off on a water bath maintained at 70° C. and the extract dried *in vacuo* for 3 hr. at 98 to 100° C. at a pressure not exceeding 25 mm. mercury. The entire technique was carefully standardized in order to avoid differences in treatment.

The expressed oils were prepared by pressing 25 gm. of the ground samples, at room temperature, in a special $2\frac{1}{4}$-in. cylinder at a pressure of approximately 16,000 lb. per sq. in. in the Carver laboratory hydraulic press. A filter pad of Whatman No. 4 paper placed at the bottom of the press cylinder obviated the necessity of filtering the expressed oil. Sufficient oil for the refractometric and iodine value determinations was obtained after five to ten minutes' pressing; this was collected in small glass vials and the above tests carried out on the same day. Preliminary experiments revealed that variations in pressure from 1,000 to 20,000 lb. per sq. in. and in pressing time from 3 min. to 1 hr. at room temperature were without influence on the refractive index or iodine value of the expressed oil. It was found, however, that a slight increase in refractive index resulted when the hot plates between which the press cylinder is placed, were maintained at temperatures in excess of approximately 70° C.

The refractive indices of the oils were determined at 25° C. with a Zeiss refractometer equipped with interchangeable water-jacketed prism heads, as described in a previous paper (12); the "linseed oil" prism with arbitrary scale values of −5.0 to 105.0 corresponds to refractive indices of from 1.48986 to 1.46514.

Iodine values were determined in duplicate by the Wijs method according to the official A.O.A.C. (3) method, chloroform being used as the solvent; this method calls for a sample weight of 0.1 to 0.2 gm. oil and a contact time of 30 min. in the dark. There is a considerable lack of uniformity in the specifications given by different authorities for conducting the Wijs method and several studies have shown that variations in excess reagent, time and temperature of reaction affect the iodine value. The specifications of the American Society for Testing Materials (1) correspond closely to those of the A.O.A.C.; on the other hand, the Federal Specifications Board (7) specify the use of an 0.09 to 0.15 gm. sample and a reaction time of 1 hr. at 21 to 23° C. for raw linseed oil.

The refractometric and iodine values for the extracted and expressed oils prepared from the 110 samples of flaxseed were submitted to statistical analysis, the results of which are summarized in Table I.

TABLE I

STATISTICAL CONSTANTS FOR DATA ON REFRACTOMETRIC SCALE READING AND IODINE VALUE OF ETHER EXTRACTED AND COLD PRESSED OILS PREPARED FROM 112 SAMPLES OF FLAXSEED

——		Mean	Min.	Max.	S.D.	C.V.
Refractometric scale reading at 25° C.*						
Ethyl ether extract	*(a)*	46.63	37.4	60.0	5.36	11.49
Cold pressed oil	*(b)*	48.37	37.6	62.3	5.87	12.14
Iodine value (Wijs)						
Ethyl ether extract	*(x)*	176.66	151.2	196.0	10.71	6.06
Cold pressed oil	*(y)*	178.88	152.6	200.7	11.02	6.16

SIMPLE CORRELATION COEFFICIENTS

	r	5% point
r_{ab}	0.9498	0.195
r_{ax}	− .9188	.195
r_{by}	− .9781	.195
r_{xy}	.9529	.195
r_{ay}	− .9392	.195

REGRESSION EQUATIONS AND STANDARD ERRORS OF PREDICTION

Iodine value (ethyl ether extract) on refractometric scale reading ethyl ether extract:— $x = 262.29 - 1.8364a$. Standard error of prediction, 4.18.

Iodine value (cold pressed oil) on refractometric scale reading (cold pressed oil):— $y = 267.64 - 1.8351b$. Standard error of prediction, 2.31.

(*) *The refractive indices corresponding to the tabulated scale readings are as follows:—*

	Mean	*Min.*	*Max.*
Ethyl ether extract	*1.47890*	*1.48094*	*1.47587*
Cold pressed oil	*1.47851*	*1.48090*	*1.47534*

For simplicity and convenience, the calculations were made on the refractometer scale readings without conversion to refractive indices; and in interpreting the statistics, it should be borne in mind that scale reading is inversely related to refractive index. The mean refractive index of cold pressed oil is lower and the mean iodine value higher than the corresponding values for the ether-extracted oil; as the refractive index and iodine value of both the extracted and expressed oils are positively correlated, this implies that the two oils are not strictly comparable in character. The correlation of 0.9781 between these variables for the cold pressed oils is significantly higher than the corresponding statistic 0.9188 for the ether-extracted oils; moreover, the correlation 0.9392 between the refractive index of ether-extracted oil and the iodine value of the cold pressed oil is significantly lower than the above value 0.9781, which indicates that the refractive index of the ether-extracted oil has been altered in an irregular manner by the processes involved in its preparation. As will be shown later, this may be ascribed to the sensitivity of the refractive index of linseed oil to unavoidable variations in the heat treatment to which it is subjected. It is of interest to note, however, that the correlation 0.9188 between refractive index and iodine value for the ethyl ether extracts is considerably higher than that previously reported by the authors (12), namely, 0.647; this is presumably due to the special precautions taken to standardize the technique employed in the present study for the preparation of the extracts. In evaluating different lots of flaxseed, it is desirable to obtain the oil in an essentially unchanged condition and this is most closely approached by cold pressing. This method is also preferable from the standpoint of cost, convenience and time and the greater accuracy with which iodine value may be predicted.

TABLE II

STATISTICAL CONSTANTS FOR REFRACTOMETRIC SCALE READING AND IODINE VALUE OF COLD PRESSED OILS PREPARED FROM 339 SAMPLES OF FLAXSEED

Statistic	Refractometric* scale reading, 25° C. (b)	Iodine value (Wijs) (y)
Mean	44.77	185.68
Minimum	37.0	152.6
Maximum	62.3	201.7
S. D.	5.64	10.38
C. V.	12.62	5.54

Correlation—Scale reading x iodine value $r_{by} = -0.980$.
Regression of iodine value on refractive index $y = 266.18 - 1.7980b$.
Standard error of prediction of iodine value = 2.08.

* *The refractive indices (n_D^{25}) corresponding to the mean, minimum and maximum scale readings are 1.47931, 1.48125 and 1.47534 respectively.*

In view of these considerations, the cold pressed oils from an additional 227 samples were analyzed in order to secure a more reliable basis for establishing the conversion equation for the estimation of iodine value from the refractometric scale readings. The results for the entire series of 339 samples are summarized in Table II. The correlation obtained between scale reading

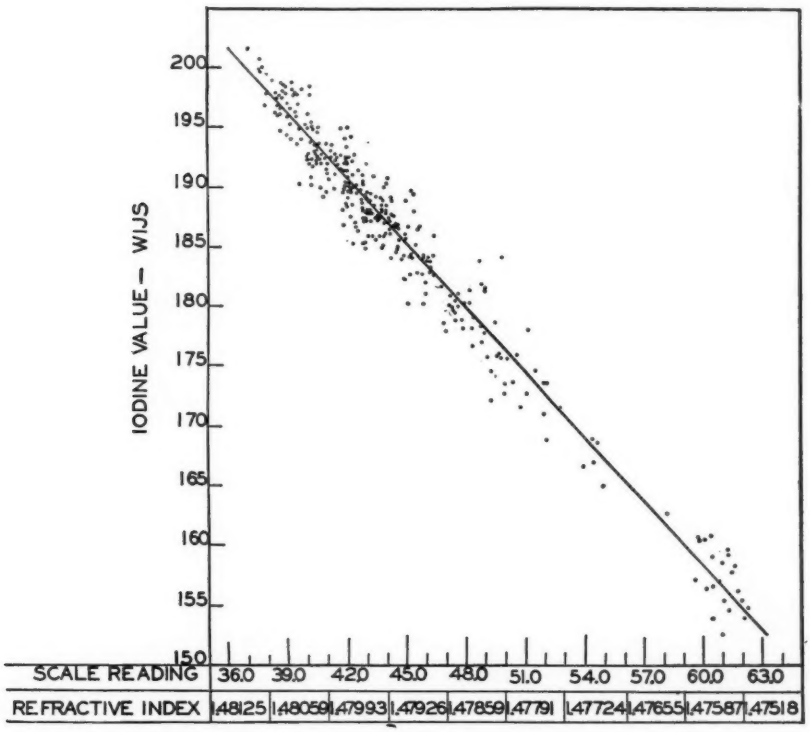

FIG. 1. *Regression of iodine value on Zeiss refractometric scale reading for cold pressed linseed oils.*

and iodine value of -0.980 permits prediction of iodine value from the regression equation with a standard error of prediction of 2.1 units, as compared with a correlation of -0.919 and a standard error of 4.2 units for the ether-extracted oils from 112 samples. The relation between these variables is illustrated graphically in Figs. 1 and 2 for the expressed and extracted oils respectively, and a conversion chart for estimating iodine values from the refractive indices of cold pressed oils is given in Table III.

As an index of the reliability of the refractometric method for estimating iodine value, it is of interest to compare these data with those recently published by Zeleny and Coleman (22, 23) and Hopper and Nesbitt (13).

TABLE III

CONVERSION CHART FOR COMPUTING WIJS IODINE VALUE OF COLD PRESSED OIL FROM ZEISS REFRACTOMETER SCALE READING AND CORRESPONDING REFRACTIVE INDEX*

Refractive index, n_D^{25}	Zeiss scale reading at 25° C.	Iodine value (Wijs)	Refractive index, n_D^{25}	Zeiss scale reading at 25° C.	Iodine value (Wijs)	Refractive index, n_D^{25}	Zeiss scale reading at 25° C.	Iodine value (Wijs)	Refractive index, n_D^{25}	Zeiss scale reading at 25° C.	Iodine value (Wijs)	Refractive index, n_D^{25}	Zeiss scale reading at 25° C.	Iodine value (Wijs)
1.48147	35.0	203.2	1.48048	39.5	195.2	1.47950	43.9	187.2	1.47852	48.3	179.3	1.47753	52.7	171.4
1.48145	35.1	203.1	1.48046	39.6	195.0	1.47948	44.0	187.1	1.47850	48.4	179.2	1.47751	52.8	171.2
1.48143	35.2	202.9	1.48044	39.7	194.8	1.47946	44.1	186.9	1.47848	48.5	179.0	1.47748	52.9	171.1
1.48140	35.3	202.7	1.48041	39.8	194.6	1.47944	44.2	186.7	1.47845	48.6	178.8	1.47746	53.0	170.9
1.48138	35.4	202.5	1.48039	39.9	194.4	1.47941	44.3	186.5	1.47843	48.7	178.6	1.47744	53.1	170.7
1.48136	35.5	202.4	1.48037	40.0	194.3	1.47939	44.4	186.3	1.47841	48.8	178.4	1.47742	53.2	170.5
1.48134	35.6	202.2	1.48035	40.1	194.1	1.47937	44.5	186.2	1.47838	48.9	178.3	1.47739	53.3	170.3
1.48132	35.7	202.0	1.48033	40.2	193.9	1.47935	44.6	186.0	1.47836	49.0	178.1	1.47737	53.4	170.2
1.48129	35.8	201.8	1.48030	40.3	193.7	1.47933	44.7	185.8	1.47834	49.1	177.9	1.47735	53.5	170.0
1.48127	35.9	201.6	1.48028	40.4	193.5	1.47930	44.8	185.6	1.47832	49.2	177.7	1.47733	53.6	169.8
1.48125	36.0	201.5	1.48026	40.5	193.4	1.47928	44.9	185.4	1.47829	49.3	177.5	1.47731	53.7	169.6
1.48123	36.1	201.3	1.48024	40.6	193.2	1.47926	45.0	185.3	1.47827	49.4	177.4	1.47728	53.8	169.4
1.48121	36.2	201.1	1.48022	40.7	193.0	1.47924	45.1	185.1	1.47825	49.5	177.2	1.47726	53.9	169.3
1.48118	36.3	200.9	1.48019	40.8	192.8	1.47922	45.2	184.9	1.47823	49.6	177.0	1.47724	54.0	169.1
1.48116	36.4	200.7	1.48017	40.9	192.6	1.47919	45.3	184.7	1.47821	49.7	176.8	1.47722	54.1	168.9
1.48114	36.5	200.6	1.48015	41.0	192.5	1.47917	45.4	184.6	1.47818	49.8	176.6	1.47720	54.2	168.7
1.48112	36.6	200.4	1.48013	41.1	192.3	1.47915	45.5	184.4	1.47816	49.9	176.5	1.47717	54.3	168.5
1.48110	36.7	200.2	1.48011	41.2	192.1	1.47913	45.6	184.2	1.47814	50.0	176.3	1.47715	54.4	168.4
1.48107	36.8	200.0	1.48008	41.3	191.9	1.47911	45.7	184.0	1.47812	50.1	176.1	1.47713	54.5	168.2
1.48105	36.9	199.8	1.48006	41.4	191.7	1.47908	45.8	183.8	1.47809	50.2	175.9	1.47710	54.6	168.0
1.48103	37.0	199.7	1.48004	41.5	191.6	1.47906	45.9	183.7	1.47807	50.3	175.7	1.47708	54.7	167.8
1.48101	37.1	199.5	1.48002	41.6	191.4	1.47904	46.0	183.5	1.47805	50.4	175.6	1.47706	54.8	167.6
1.48099	37.2	199.3	1.48000	41.7	191.2	1.47902	46.1	183.3	1.47803	50.5	175.4	1.47703	54.9	167.5
1.48096	37.3	199.1	1.47997	41.8	191.0	1.47899	46.2	183.1	1.47800	50.6	175.2	1.47701	55.0	167.3
1.48094	37.4	198.9	1.47995	41.9	190.8	1.47897	46.3	182.9	1.47798	50.7	175.0	1.47699	55.1	167.1

* *The values were computed from the regression equation: Iodine value = 266.18 − 1.798 (Zeiss scale reading) at 25° C.*

TABLE III—*Concluded*

CONVERSION CHART FOR COMPUTING WIJS IODINE VALUE OF COLD PRESSED OIL FROM ZEISS REFRACTOMETER SCALE READING AND CORRESPONDING REFRACTIVE INDEX*

Refractive index, n_D^{25}	Zeiss scale reading at 25° C.	Iodine value (Wijs)	Refractive index, n_D^{25}	Zeiss scale reading at 25° C.	Iodine value (Wijs)	Refractive index, n_D^{25}	Zeiss scale reading at 25° C.	Iodine value (Wijs)	Refractive index, n_D^{25}	Zeiss scale reading at 25° C.	Iodine value (Wijs)	Refractive index, n_D^{25}	Zeiss scale reading at 25° C.	Iodine value (Wijs)
1.48092	37.5	198.8	1.47993	42.0	190.7	1.47895	46.4	182.8	1.47796	50.8	174.8	1.47697	55.2	166.9
1.48090	37.6	198.6	1.47991	42.1	190.5	1.47893	46.5	182.6	1.47793	50.9	174.7	1.47694	55.3	166.8
1.48088	37.7	198.4	1.47988	42.2	190.3	1.47890	46.6	182.4	1.47791	51.0	174.5	1.47692	55.4	166.6
1.48085	37.8	198.2	1.47986	42.3	190.1	1.47888	46.7	182.2	1.47789	51.1	174.3	1.47690	55.5	166.4
1.48083	37.9	198.0	1.47984	42.4	189.9	1.47886	46.8	182.0	1.47787	51.2	174.1	1.47687	55.6	166.2
1.48081	38.0	197.9	1.47982	42.5	189.8	1.47883	46.9	181.9	1.47784	51.3	173.9	1.47685	55.7	166.0
1.48079	38.1	197.7	1.47979	42.6	189.6	1.47881	47.0	181.7	1.47782	51.4	173.8	1.47683	55.8	165.9
1.48077	38.2	197.5	1.47977	42.7	189.4	1.47879	47.1	181.5	1.47780	51.5	173.6	1.47680	55.9	165.7
1.48074	38.3	197.3	1.47975	42.8	189.2	1.47877	47.2	181.3	1.47778	51.6	173.4	1.47678	56.0	165.5
1.48072	38.4	197.1	1.47972	42.9	189.0	1.47874	47.3	181.1	1.47776	51.7	173.2	1.47676	56.1	165.3
1.48070	38.5	197.0	1.47970	43.0	188.9	1.47872	47.4	181.0	1.47773	51.8	173.0	1.47674	56.2	165.1
1.48068	38.6	196.8	1.47968	43.1	188.7	1.47870	47.5	180.8	1.47771	51.9	172.9	1.47671	56.3	165.0
1.48066	38.7	196.6	1.47966	43.2	188.5	1.47868	47.6	180.6	1.47769	52.0	172.7	1.47669	56.4	164.8
1.48063	38.8	196.4	1.47963	43.3	188.3	1.47866	47.7	180.4	1.47767	52.1	172.5	1.47667	56.5	164.6
1.48061	38.9	196.2	1.47961	43.4	188.1	1.47863	47.8	180.2	1.47764	52.2	172.3	1.47664	56.6	164.4
1.48059	39.0	196.1	1.47959	43.5	188.0	1.47861	47.9	180.1	1.47762	52.3	172.1	1.47662	56.7	164.2
1.48057	39.1	195.9	1.47957	43.6	187.8	1.47859	48.0	179.9	1.47760	52.4	172.0	1.47660	56.8	164.1
1.48055	39.2	195.7	1.47955	43.7	187.6	1.47857	48.1	179.7	1.47758	52.5	171.8	1.47657	56.9	163.9
1.48052	39.3	195.5	1.47952	43.8	187.4	1.47854	48.2	179.5	1.47755	52.6	171.6	1.47655	57.0	163.7
1.48050	39.4	195.3												

* *The values were computed from the regression equation: Iodine value = 266.18 − 1.798 (Zeiss scale reading) at 25° C.*

The experimental technique and the results obtained by the three laboratories are summarized in Table IV and the agreement obtained indicates that the refractometric method of estimating iodine value is quite reliable, especially when it is considered that the methods employed for preparing the oils and determining their iodine values were not identical.

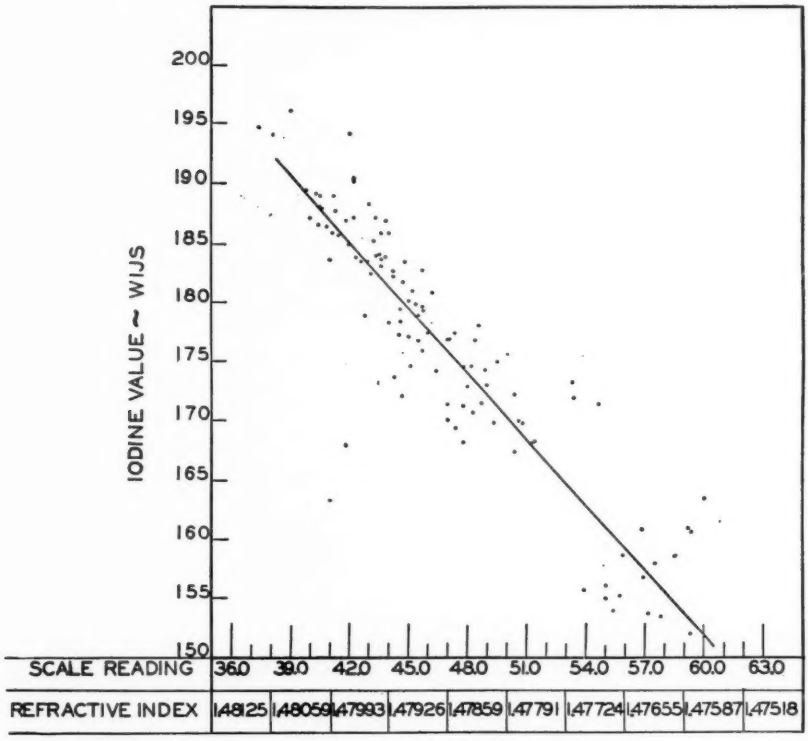

FIG. 2. *Regression of iodine value on Zeiss refractometric scale reading for ethyl ether extracted linseed oils.*

Zeleny and Coleman (22) prepared the majority of their oil samples by a partial extraction of the ground samples with petroleum ether, the extract being dried in an air oven at 105° C. for 30 min., and they present data for six samples showing that the refractive indices of oils prepared by this procedure are practically identical with those obtained by cold pressing. On the other hand, in order to conserve time and thereby render the method more applicable to routine testing, Hopper and Nesbitt (13) heat the meal in a beaker suspended in a steam bath for 20 min. and express the oil between hot plates at 60 to 70° C. They present comparative refractive indices and iodine values of oils expressed by this method with those obtained by cold

TABLE IV

COMPARISON OF THE RELATIONS BETWEEN REFRACTIVE INDEX OF RAW LINSEED OIL AND IODINE VALUE AS REPORTED BY DIFFERENT WORKERS

——	Zeleny and Coleman	Hopper and Nesbitt	Lehberg and Geddes
Method of preparing oil	Either by cold pressing in a laboratory hydraulic press at 16,000 pounds per square inch or by partial extraction with petroleum ether, and drying extract in air oven at 105° C. for 30 minutes.	Meal heated in a steam bath for 20 minutes and warm pressed between hot plates at 60 to 70° C.	Oil cold pressed from ground meal at 16,000 pounds per square inch.
Method of determining refractive index	Zeiss refractometer at 25° C.	Abbé refractometer at 25° C.	Zeiss refractometer at 25° C.
Method of determining iodine value	Wijs method according to instructions of Federal Specifications Board (reaction time 60 minutes).	Wijs method according to "official" procedure (presumably A.O.A.C. specifications) but using a reaction time of 60 minutes.	Wijs method according to A.O.A.C. specifications (reaction time 30 minutes).
Number of samples analyzed	96	1485	339
Refractive index range	1.47589 to 1.48065	1.47420 to 1.48047	1.47534 to 1.48125
Iodine value range	155.4 to 197.3	144 to 196	152.6 to 201.7
Correlation to refractive index x iodine value	0.996	0.989	0.980
Regression equation	Iodine value = 8584.97 (n_D^{25}) − 12513.83	n_D^{25} = 1.45723 + .00011846 (iodine value)	Iodine value = 266.18 − 1.7980 (Zeiss scale reading).
Standard error of estimate of iodine value from refractive index	0.8	1.5	2.1

COMPARISON OF CONVERSION CHARTS

Refractive index n_D^{25}	Estimated iodine value (Wijs)		
	Zeleny and Coleman	Hopper and Nesbitt	Lehberg and Geddes
1.4750	149.0	149.4	151.5
1.4755	153.3	154.2	155.4
1.4760	157.6	158.4	159.4
1.4770	166.2	166.9	167.3
1.4780	174.8	175.3	175.2
1.4790	183.3	183.8	183.1
1.4800	191.9	192.2	191.2
1.4810	200.5	200.7	199.5
1.4817	206.5	206.6	205.0

pressing on 26 samples of flaxseed. The mean iodine value of the warm pressed oils was 0.1 units higher and the mean refractive index 0.00007 units higher than for the cold pressed oils, and they conclude that in the majority of the samples the differences are within the limits of reasonable experimental error. On casual examination of their results, we noted that the refractive index of the warm pressed oils exceeded that of the cold pressed in 18 out of 26 cases, the differences ranging as high as 0.00026 units. Accordingly, a statistical analysis of their data was made which showed that, while the mean iodine values of cold and warm pressed oils were not significantly different, the refractive index of the warm pressed oils was significantly higher.

As mentioned earlier in this paper, the writers also found the refractive index of the oil to be increased slightly when the oil was prepared by pressing between hot plates maintained above approximately 70° C.; this would account for the slightly higher predicted iodine values reported by the other workers.

That the use of heat in preparing the oils is undesirable is substantiated by experiments we have conducted in which cold pressed linseed oil has been heat-treated for 30 min. in the presence of air at 25° C. increments of temperature from 25° to 200° C. The refractive indices began to increase at approximately 75° C., while the iodine values remained substantially unaltered until a temperature of approximately 125° C. was attained.

These results serve to explain the closer degree of association between refractive index and iodine value of cold pressed as compared with ether-extracted oils found by the writers, and clearly indicate the desirability of preparing the oils by cold pressing, as this procedure is the least likely to induce changes in physical and chemical characteristics.

Discussion

The rapid optical procedure for estimating iodine value, in conjunction with the improved refractometric method previously described by the authors, has been employed in flaxseed studies carried out by this laboratory since January, 1936, and has been found to be of great utility in conducting surveys of the quality of flaxseed produced in different districts of Western Canada and in evaluating new varieties and hybrids submitted by plant breeders, where the greatest accuracy is not required. The method is not applicable to flax that is musty or heated, although it has been found to give reliable results with immature, frost damaged or scabby flax. Zeleny and Coleman (22, 23) have made similar observations in this connection and point out that the method is intended to apply only to samples of oil from freshly ground unaltered material. The refractometric method obviously cannot be employed on commercially prepared linseed oils but should be of considerable utility to linseed crushers in securing a measure of the intrinsic drying values of their raw material.

Acknowledgment

The authors are indebted to Miss Nancy Milton, Statistical Assistant, Associate Committee on Grain Research, for her valuable assistance in carrying out the statistical calculations.

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INHERITANCE OF QUALITY AND QUANTITY OF OIL IN FLAX IN RELATION TO OTHER PLANT CHARACTERS[1]

By W. G. McGregor[2]

Abstract

Data from 21 varieties of flax, grown at the Central Experimental Farm over a five-year period, were analyzed statistically to determine the relation of quantity and quality of oil to seed size, days to maturity, days from flowering to maturity, and height of plant. In addition, hybrids of Cyprus × Ottawa 770B and Buda × Ottawa 770B were studied to determine the genetic basis for the inheritance of quality of oil, flower type, color of seed, color of oil, seed size and height of plant.

The refractometric method for determining the quantity and quality of oil was compared with the ether extraction method for oil content and the Wijs method for iodine number and found to be very practical for breeding studies.

In the variety test, high oil content was associated with a long period from blossoming to maturity and with large-seeded varieties.

In the hybrids, iodine number, seed size, and height of plant are apparently dependent on several genetic factors. No significant association between oil content, iodine number, seed size or height of plant was found among these hybrids. The inheritance of flower and seed type has been explained on the basis of a single factor, the Ottawa 770B type with white, narrow, involute petal and greenish-yellow seed being inherited as a simple recessive or the expression of several very closely linked recessive genes. An association of high iodine number with this factor for yellow seed color was indicated in both hybrids.

Although insufficient data were collected to give definite conclusions, evidence indicated that color of oil, as measured by carotinoid pigment content, had a genetic basis. No correlation was indicated between carotene pigment content and the quantity and quality of the oil or color of the seed.

Introduction

Heretofore, improvement in flax has been based largely on yield and on disease resistance, especially resistance to wilt. Reasonable advances in attaining these qualities have already been accomplished so that the next problem is to improve the yield of oil and its drying qualities. Flaxseed is purchased primarily for the oil it contains, which represents from two-thirds to three-fourths of the value of the manufactured products. Methods of oil determination have already been established which, if adopted for commercial purposes, will put a premium on the quantity and quality of the oil present in any market sample. A recognition of quality on the official inspection certificate would be an added incentive to the farmer and would create a demand for varieties of high quality.

Yellow-seeded varieties of flax have been observed to be high in quality. A variety of the yellow-seeded type would appear to have other advantages over the more common brown-seeded varieties, *viz.*, in the production of a lighter colored oil cake and especially a lighter colored oil. In a flax improvement program it is necessary to have a knowledge of the manner in which these characters are inherited and their relation to one another.

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[2] *Cerealist. Flax investigations.*

Literature Review

Studies in Flax

Inheritance of characters in flax has been previously studied and reviewed by Tammes (21, 22) and Shaw, Khan and Alam (20).

Many early investigators have made observations on the relation of various kernel characters to the chemical composition of flaxseed. Eyre and Fisher (8), Birchard (1), Rabak (19) and Geddes and Lehberg (10) observed that in practically all cases an increase in oil content accompanied an increase in size of the seed. Coleman and Fellows (4) in a more extensive study considered test weight per bushel, color and size of seed. Oil content was found to increase gradually as the kernels grew larger. It was pointed out, however, that this relation is not specific and that small plump seeds often contain as great a percentage of oil as a sample containing very large kernels. No relation was found between depth of seed color and oil content.

Johnson (14), in a statistical study, found significant simple correlations between the oil content and weight per 1000 kernels, date of maturity and number of days from full bloom to maturity. A high multiple correlation between percentage of oil and the above variables was taken to indicate that the total effect of these characters determines to a considerable extent the percentage of oil. Significant inter-annual correlations of oil content, seed weight, days to maturity and the period from full bloom to maturity indicated that these characters are relatively constant in their inheritance. A low multiple correlation of iodine number with these characters was interpreted as suggesting that iodine number was inherited independently of the plant and seed characters studied.

Other investigators have not agreed with these findings on the relation of size of seed to oil content. Leather (16) analyzed samples of flaxseed collected from several provinces in India and concluded there was no relation between size of seed and oil content. More recently Ermakov (6) used a technique in which individual seeds were analyzed. The correlations observed were occasionally negative, and in seeds giving the same oil content one was often twice the weight of the other and *vice versa*. These differences were ascribed to the genetical nature of the different seeds.

The iodine number, as an index of drying quality, has been studied in relation to oil content and certain other characters. Johnson (14) found a significant negative correlation of -0.309 between iodine number and weight per 1000 seeds. Dillman (5) has reported the work of Arny who explained the inheritance on the action of a single factor with low iodine number dominant. Yellow seed-coat appeared to be linked with high or medium iodine number.

Related Studies in Other Oil Bearing Seeds

A study of the chemical composition of corn was begun in 1896 at the Illinois Agricultural Experiment Station (25). After 28 years of continuous selection for high and low oil content, and high and low protein content, four types were produced which were distinctly different in composition. Selection was more effective in bringing a change in oil content than in protein content.

It was suggested that the primary materials from which fat is manufactured, namely, carbon dioxide and water, are usually furnished to the plant in unlimited supply, while the formation of protein is essentially dependent upon the supply of available nitrogen in the soil.

An experiment similar to that with corn was started with soybeans by Woodworth (26) in 1914. While selection within pure lines was ineffective either in the high or low direction, the F_1 hybrids between high and low lines were intermediate and the F_2 showed transgressive segregation. No attempt was made to estimate the number of genes which were responsible for the difference between the high and low strains.

An experiment was started at the Wisconsin Station in 1912 by Cole, Lindstrom and Woodworth (3) to determine whether any progress could be made in selection of soybeans for quality of the oil. After seven years' selection the quality and quantity of oil showed a high degree of variability but no consistent relation. Indirect evidence and some experimental data indicated that high quality was correlated with late maturity. Their data indicate that genotypes were isolated for type of oil and other characteristics.

Recently increased interest has been shown in the oil content of sunflowers, ground nut, and numerous other plants which have possibilities in the production of oil. Ermakov (6) states that Hildebrandt has obtained a correlation as high as 0.66 ± 0.07 between oil content and color of seed in sesamum. Ermakov found it possible to eliminate lines of low oil content in sunflowers with improved methods of technique but observed no relation between color of seed and oil content. More recently Ermakov *et al.* (7) have studied the intergeneric and interspecific differences in oil content of lupine seeds. A wide variation in oil content was found in this material.

Method of Procedure

Since scarcely any information was available regarding the oil content and quality of flax varieties grown in Canada, a survey of the varieties grown at the Central Experimental Farm, Ottawa, Canada, was made during the years 1929 and 1931-1934 inclusive. A number of foreign and domestic linseed varieties including fibre types were considered in this test. They were grown in a quadruplicate rod-row test arranged systematically, and all plots of each variety were bulked for analysis of oil content and iodine number.

Characters studied in relation to oil content and iodine number included height of plant, weight per 1000 kernels, weight per measured bushel, days to maturity, length of period from full bloom to maturity, and yield. Analyses of variance and correlations with these characters for the 21 varieties over the five-year period were made in accordance with methods outlined by Goulden (13).

Hybridization studies were begun in 1933. Varieties from which some data on the oil content and iodine number were available, as ascertained from the varietal survey, were used. Although several combinations were made,

only two, Ottawa 770B × Cyprus and Ottawa 770B × Buda, have been studied in detail. The description of these varieties follows:—

Cyprus C.A.N. 2110* is short-strawed with a blue, rather flat flower, and medium broad petals. The anthers, filament, stigma and style are blue. The seed is brown and very large, averaging 9.2 gm. per 1000 kernels at this station. In maturity it is medium, blossoming very early and having a long period from blossoming to maturity. The oil content over a five-year period averaged 39.5 ± 1.94% and the iodine number 170.0 ± 7.56.

Buda C.A.N. 2104 has a smaller, somewhat lighter blue, tubular to funnel-shaped flower. The anthers are blue with light blue filaments. The style is deep blue with a mauve to purple stigma. The seeds are brown and very small, averaging 4.1 gm. per 1000 kernels. The oil content over a five-year period averaged 38.1 ± 1.71% and the iodine number 186.2 ± 4.17.

Ottawa 770B, C.A.N. 2152, has a white flower with a narrow involute petal. The anthers are yellow, the filament and stigma are white, the style being light yellow at the base and white at the tip. The seeds are greenish-yellow, of medium size, averaging 6.0 gm. per 1000 kernels. In maturity it is very late. The oil content averaged 41.2 ± 0.80% and the iodine number 187.7 ± 5.34.

These hybrids were made in the field during the summer of 1933 and the F_1 generation was grown in the greenhouse during the winter of 1933–34. The F_2 was grown in the field in 1934. Individual F_2 plants were tagged and notes taken on the shape and color of the floral parts. The plants were pulled when mature and taken to the laboratory where each plant was examined as to height and color of seed. The third generation was grown in the field during the season of 1935. Forty-eight seeds of each F_2 line chosen were seeded in two rows, each four feet long. Fifty lines of each parent were seeded in a similar manner in alternate plots. The F_3 lines were pulled and dried in the drying barn after which they were examined and threshed. Each sample was handpicked before taking weight per 1000 kernels and making an oil analysis. Weight per 1000 kernels was determined by counting 250 seeds at random in duplicate and weighing to ± 0.1 gm.

Oil determinations were made by both the ethyl ether extraction method and the improved refractometric method as described by Geddes and Lehberg (11).

A refractometric method for measuring quality, developed by the Grain Research Laboratory, was also used. A sample of ground, undried flax pulp was placed in the pressing cylinder of a Carver laboratory press and subjected to a pressure of approximately 16,000 lb. to the square inch for 10–15 min. and the refractive index of the oil determined. The iodine number was also obtained by the Wijs method.

Studies have shown a very close relation between the refractive index o the cold pressed, untreated, linseed oil and the Wijs iodine value. Data fro a

* *C.A.N. = Canadian Accession Number.*

the Grain Research Laboratory on a large number of samples covering many different varieties and types of flax have given a correlation coefficient of 0.980. In the work on this problem, when only 25 samples of each parent were analyzed, the correlation coefficient in each case was highly significant. Owing to the time required for making quality tests by the Wijs iodine method, the refractive index only was run on samples of Buda and the hybrid Buda × Ottawa 770B.

Color intensity was studied spectrophotometrically by a method used in the Grain Research Laboratory. This is a modification of the method for flour outlined by Geddes, Binnington and Whiteside (12). Four grams of undried flaxseed pulp, on a dry basis, was weighed into two 150 cc. glass-stoppered bottles to which were added, from a pipette, 100 cc. of a mixture of 93 parts by volume of a petroleum distillate, known commercially as varnish makers' naphtha, with seven parts by volume of absolute ethyl alcohol. The bottle was shaken for two hours in a mechanical shaker and the supernatant liquid decanted into 100-cc. lipless centrifuge tubes, closed with a rubber cap and centrifuged at approximately 2000 r.p.m. for 30 min. The clear extract was siphoned off with a capillary bore siphon, and the transmittancy determined in a 10 cm. cell at a wave-length of 435.8Å, a mercury arc being used as a light source. By this method the carotinoid and similar pigments are extracted. However, throughout this study they have been referred to as "carotene".

Survey of Varieties

Characters of a variety or strain, such as size of seed, height of plant, maturity and yield, might be expected to bear some relation to the quantity and quality of the oil produced. An analysis of variance of oil content, iodine number and size of seed, of the varieties and strains of flax that were tested throughout a five-year period is given in Table I. While these 21 lines include a fair sample of the leading varieties of flax, a period of five years is not as representative of the seasons as might be desirable. It will be noted in this table that there is a significant variation, as shown by the F value, for each character studied. Season as well as variety has a significant influence

TABLE I

ANALYSIS OF VARIANCE OF OIL CONTENT IN PER CENT, IODINE NUMBER AND WEIGHT PER THOUSAND KERNELS IN GRAMS

Source of variation	Degrees of freedom	Oil content		Iodine number		Size of seed		1% point
		Mean square	F value	Mean square	F value	Mean square	F value	
Total	104	4.331	4.6	46.19	3.8	1.68	9.7	1.47
Between years	4	25.655	27.1	540.34	44.9	2.40	13.9	3.56
Between varieties	20	13.604	14.4	890.09	74.0	75.91	43.9	2.03
Remainder	80	.947		12.03		.17		

on the oil content, iodine number and size of seed. The data on these characters for each year are given in the original thesis, but since space will not permit their publication here the average has been given in Table II.

TABLE II

AVERAGE DATA ON FLAX VARIETIES GROWN AT THE CENTRAL EXPERIMENTAL FARM FOR THE YEARS 1929 AND 1931–34 INCLUSIVE

Variety	Canadian Accession Number	Oil content, %	Iodine number	Wt. per 1000 K, gm.	Yield per acre, bu.	Days to maturity	Days from flowering to maturity	Length of straw, in.
Buda	2104	38.1	186.2	4.1	19.4	92.8	37.9	21.2
Chippewa	2105	38.8	179.0	4.8	18.2	95.7	39.3	22.4
Damask	2111	38.7	187.5	4.1	14.1	94.4	37.1	23.9
Diadem	2113	42.5	180.1	6.6	16.7	90.8	42.1	19.5
J. W. S.	2117	39.9	183.6	4.3	14.2	92.9	38.5	28.8
Linota	2120	38.6	188.5	4.4	16.9	94.0	39.7	23.2
N. D. R. 52	2130	38.8	184.6	4.3	18.7	93.1	39.3	23.2
N. D. R. 114	2131	38.9	183.2	4.9	17.4	91.0	35.6	22.9
Novelty	2135	41.9	178.5	5.4	18.7	94.8	41.5	22.3
Palermo	2136	41.3	179.9	7.1	18.1	98.0	45.2	22.2
Redwing	2140	40.1	184.6	4.7	19.5	93.3	40.4	22.0
Siberian	2142	40.8	182.3	4.4	19.0	95.6	39.3	22.0
Slope	2143	38.9	188.6	4.4	18.4	91.9	39.5	21.2
Slope 585	2144	39.3	183.8	4.7	18.0	92.4	38.3	22.3
Winona	2146	38.5	185.1	4.5	19.1	94.7	39.8	22.2
8B	2147	42.9	175.3	7.4	19.9	104.3	48.0	18.1
8C	2148	43.4	179.5	7.3	19.7	103.7	48.5	18.9
9B	2149	41.8	177.2	6.8	19.0	101.2	47.8	19.7
12C	2150	42.9	179.2	6.7	19.8	102.8	49.4	18.6
12C42	2151	41.5	174.7	7.1	18.3	104.8	51.4	19.8
Ottawa 770B	2152	41.2	187.7	5.5	16.8	102.8	43.3	22.1

The relation of quality or iodine number to the percentage of oil in the seed as measured by the correlation coefficient is shown in Table III. The high negative correlation between oil content and iodine number for varieties would indicate that varieties that yielded a good quantity of oil might be expected to yield oil of poor quality. However, the partial correlation of the fourth order, calculated from the correlations between varieties, considering size of seed, height, yield and days from full bloom to maturity, was -0.068. This would indicate that were varieties considered that were similar in size, yield, height and days from full bloom to maturity, no relation would be found between oil content and iodine number. Johnson (14) reports an analogous relation between these characters in flax in which a simple negative correlation of -0.056 was changed to a positive partial correlation of 0.272.

A highly significant positive correlation between oil content and size of seed is indicated, both for varieties and for years, in Table IV. In the case of iodine number, only the negative correlation for varieties is significant. Size of seed varies more with variety than with season, while oil content is influenced by season as well as variety. The partial correlation of the fourth order between size of seed and oil content is 0.259. This may indicate that under conditions in which the varieties would have the same iodine

number, yield, height and days from flowering to maturity, no significant relation would be expected between oil content and seed size. Many investigators (1, 5, 8, 10, 14), dealing with varieties or commercial samples, have noted that in practically all cases an increase in oil content accompanied an increase in the size of seed.

TABLE III

CORRELATION OF IODINE NUMBER WITH OIL CONTENT, SIZE OF SEED, MATURITY AND HEIGHT OF PLANT

Source of variation	Degrees of reedom	Iodine number				
		Oil content	Size of seed	Days to maturity	Days flowering to maturity	Height of plant
Total	103	−0.391**	−0.445**	−0.485**	−0.422	0.377**
Between years	3	− .259	− .074	− .548	− .334	.663
Between varieties	19	− .717**	− .782**	− .622**	− .730**	.496*
Remainder	79	.172	.079	− .078	− .076	− .246*

* *Significant at 5% level.*
** *Significant at 1% level.*

The question of early maturity is one of the most important features of crop improvement in Canada. Notes were taken on both the number of days to maturity and the length of the period from full bloom to maturity. From the data presented in Table III, both days to maturity and days from flowering to maturity are negatively correlated with iodine number. Oil content, however, appears more closely associated with the length of the seed-forming period than the full length of the growing period. This agrees with data presented by Johnson (14). The correlations between years in days to maturity and days from flowering to maturity are highly significant in the instance of oil content but not of iodine number. Seasons in which the ripening period is extended have a very favorable effect on the oil content but do not appreciably affect iodine number. High iodine number is related to varieties which are slow in maturing.

TABLE IV

CORRELATION OF OIL CONTENT WITH SIZE OF SEED, MATURITY AND HEIGHT OF PLANT

Source of variation	Degrees of freedom	Oil content			
		Size of seed	Days of maturity	Days flowering to maturity	Height of plant
Total	103	0.742**	0.639**	0.757**	−0.393**
Between years	3	.963**	.918**	.958**	.237
Between varieties	19	.856**	.251	.874**	− .567**
Remainder	79	.135	.042	.130	− .267*.

* *Significant at 5% level.*
** *Significant at 1% level.*

The varieties investigated were grown in a replicated yield test each season. No relation is indicated between yield per acre and oil content either between varieties or between years. A negative correlation, -0.430, appears between iodine number and yield for varieties. As the 5% point is 0.423 this association is not convincingly significant.

The 21 varieties under investigation included several short- and long-strawed types. The relation of length of straw to oil content and to iodine number are both indicated. A positive correlation is found between height and iodine number but this is not highly significant. A more significant association is that between tall varieties and low oil content.

Multiple correlations for the percentage of oil and iodine number with the other variables such as size of seed, yield, height and days from flowering to maturity, indicate the extent to which their total effect has influenced either of these characters. The multiple correlation of oil content is 0.892 and highly significant. These variables would appear, therefore, to be of value in predicting oil content. The multiple correlation of iodine number with these variables, including oil content, is 0.825 and without oil content this correlation is 0.824. This would indicate that while these variables exert a significant influence on iodine number, oil content adds little to their effect.

Inheritance Studies

Quality and Quantity of Oil

The parents used in this study varied in iodine number (Table V). Reciprocal crosses of Cyprus, a low quality variety, and Ottawa 770B, a medium high quality variety, gave F_3 progenies with iodine number ranging between the parents with few as high as Ottawa 770B and few as low as the higher lines of the Cyprus parent. The F_3 distribution in these crosses shows a slight but not significant skewness toward that of the high quality parent, the value of the g_1 statistic being -0.447 ± 0.288.

In the hybrid 33-217, in which Ottawa 770B is crossed with Buda which has high quality, the F_3 progenies again show greater variability than the parents. The distribution approaches that of a normal curve with a value for g_1 of 0.180 ± 0.312. Although a similar result might be obtained from environmental effects only, these hybrids were grown on a very small area and all lines may be expected to have been exposed to almost similar conditions. The distribution would suggest that quality is governed by several factors with no dominance. These data do not agree with those of Arny who, as reported by Dillman (5), has explained the inheritance of quality in linseed oil on the basis of a single factor with low quality dominant.

The oil content of Cyprus and Ottawa 770B did not differ as much as did iodine number. The Cyprus parent averaged $42.5 \pm 0.09\%$ of oil and the Ottawa 770B parent $41.5 \pm 0.09\%$. The oil content of the reciprocal hybrid F_3 progenies averaged 41.2 ± 0.14 and $41.9 \pm 0.11\%$ respectively.

TABLE V

FREQUENCY DISTRIBUTION OF IODINE NUMBER FOR PARENTS AND F_2 LINES

Parent or cross	No. lines	Classes for iodine number																			S.D.	C.V.
		152.5	155.0	157.5	160.0	162.5	165.0	167.5	170.0	172.5	175.0	177.5	180.0	182.5	185.0	187.5	190.0	192.5	195.0	197.5		
Cyprus	22	4	6	6	2	2	1														3.41	2.15
Ottawa 770B	25														7	6	10	2			2.16	1.14
Buda	24																	1	18	5	1.36	0.70
33-215 Ott. 770B × Cyprus	35					1	1		1	2	3	10	5	7	2	3					5.52	2.92
33-216 Cyprus × Ott. 770B	34						2	1	4	5	4	5	5	4	2	2					5.92	3.32
33-217 Ott. 770B × Buda	58													6	9	12	9	12	4	6	4.42	2.32

Flower and Seed Type

The characters of the flower and seed of the parents, as already described, differed greatly. The F_1 from the cross of Cyprus × Ottawa 770B resembled the Cyprus parent. The segregation observed in the F_2 generation was 35 blue, Cyprus type; 10 white, Ottawa 770B type, and in the reciprocal 62 blue, Cyprus type; 16 white, Ottawa 770B type. The deviations from expected, on the basis of a major factor differentiating these two types, were 1 and 3 respectively which are well within the error expected. Twenty-one selections with white flower were grown in the F_3 generation and bred true for the Ottawa 770B type. Forty-eight selections from among the blue-flowered segregates were grown, 17 of which bred true and 31 segregated as in the F_2 generation.

In the cross Buda × Ottawa 770B, the F_1 generation was similar in all respects to the Buda parent. The F_2 generation segregated as follows:— 259 blue, Buda type : 77 white, Ottawa 770B type; and the reciprocal 86 blue, Buda type : 28 white, Ottawa 770B type. The deviations in this cross are again well within the error to be expected on the basis of a monohybrid segregation. Thirty-nine selections with white flower bred true for flower type and, of 56 selections with blue flower, 18 bred true and 38 segregated as in the F_2 generation.

A deficiency of white progeny in the F_2 generation when crossing a white crimped-petal variety with blue flax has been reported by Tammes (21) and Myers (18). Tammes has attributed this deficiency to a semi-lethal effect of one of the basic factors for petal color. It has also been demonstrated by Kappert (15) that a white crimped-petal type may give a deficiency when crossed with certain blue types but not with others. In the F_2 of the crosses reported here, it will be noted that although the recessive class is smaller in each case than the theoretical, it is still well within the deviation expected on the basis of random sampling.

Yellow anthers and yellow seeds were always found associated with the white crimped-petal segregates while no blue-flowered segregates had the crimped petal. These results may be explained by assuming a basal factor which controls the color in the petal, anther, filament, style and stigma and also the color of the seed as well as the flatness or crimpiness of the petal, or several other closely linked factors. This situation has also been reported by Myers (18) in using Ottawa 770B in crosses with Redwing.

Color of Oil

The Ottawa 770B variety was used in this project because it combined high iodine value with yellow seed of very low carotene pigment content. The average of the lines tested from the 1935 crop was 11.6 ± 0.6 parts per million of pigment expressed as "carotene" while the Buda parent averaged 17.9 ± 0.6 p.p.m.

Slight variations in the depth of color of the yellow seeds were noticed among the lines selected but these differences were not so apparent among the brown types. During the pressing of these samples with the Carver

TABLE VI

FREQUENCY DISTRIBUTION OF WEIGHT PER THOUSAND KERNELS IN GRAMS OF PARENTS AND F_3 LINES

Parent or cross	Classes for weight per thousand kernels in grams																												
	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8	8.0	8.2	8.4	8.6	8.8	9.0	9.2	9.4	9.6	9.8
Buda	2	11	12																												
Ott. 770B								1	2	4	7	25	5	5	1																
Cyprus																								2	3	2	10	18	9	7	2
Buda × Ott. 770B		5	7	16	19	15	8		3																						
Buda × Cyprus							1	2		3	1	6	12	9	9	6	2	2		1											
Ott. 770B × Cyprus										1		2	4	5	12	3	10	9	9	6	4	2	2								

press, distinct differences were noticed in the color of the cold pressed oil. A number of samples were then analyzed for "carotene" pigment by the method already described. Samples differing by five parts per million yielded a cold pressed oil which showed differences in color readily discernible to the naked eye.

Light color of seed in flax has been shown by Tammes (21) to be due wholly or partly to the cotyledon showing through an entirely colorless seedcoat, the darker colors being due to pigment in the seedcoat. It was not surprising to find in the analysis of the hybrid lines, which have very distinct differences in the intensity of the yellow in the cotyledons, wide differences in pigment content, as measured by "carotene" pigment in parts per million. Selections among the yellow-seeded types from the cross Buda × Ottawa 770B varied from 17.6 p.p.m. to 35.9 p.p.m. with an average of 24.3. The brown-seeded types varied over almost as wide a range as did the yellow-seeded types. While only small numbers of the progeny were studied in this manner the segregation obtained indicated that this character was probably dependent on several factors for its expression.

Seed Size

Frequency distributions of the weight per thousand kernels in grams are presented in Table VI and summarized in Table VII. While the reciprocal progenies from crossing Cyprus and Ottawa 770B show greater variability than Buda crossed with Ottawa 770B or Cyprus, these reciprocals do not differ appreciably in their means and standard deviations and have been combined in Table VI.

The greater variability of the F_3 progeny means compared with the parents indicates that segregation for size factors has taken place. Although a considerable number of the F_3 hybrid lines are within the range of the smaller parent, only in three instances has the larger parent been recovered in these progenies. The distribution curves for these progenies, when calculated on an arithmetic basis, approach very closely a normal curve. The g_1 statistic for the hybrid Buda × Ottawa 770B is 0.290 ± 0.312 and for the hybrids Cyprus × Ottawa 770B and Cyprus × Buda -0.117 ± 0.288 and $-0.184 \pm$

TABLE VII

MEAN AND VARIATION IN WEIGHT PER THOUSAND KERNELS IN GRAMS OF PARENTS AND HYBRID LINES

——	No. of lines	Mean weight, gm.	S.E.	C.V.
Buda	25	4.09	.10	2.4
Ottawa 770B	50	5.99	.27	4.5
Cyprus	53	9.22	.35	3.8
33-215 Ott. 770B × Cyprus	35	7.04	.53	7.5
33-216 Cyprus × Ott. 770B	34	7.02	.56	8.0
33-217 Buda × Ott. 770B	58	4.55	.32	7.0
33-73 Buda × Cyprus	54	6.34	.49	7.8

TABLE VIII

DISTRIBUTION OF HEIGHT OF PLANT IN INCHES OF PARENTS AND F_3 LINES

Class	No. lines	Classes for height in inches																			Mean
		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
Buda	50														2	18	18	10	2			26.84±0.17
Cyprus	50					1	3	12	17	12	1	4										19.10± .19
Ottawa 770B	50									1	2	2	9	9	14	10	2	1				24.58± .20
33-215 Ott. 770B × Cyprus	35	1	1	3	6	4	8	3	3	1	2	2	1									17.06± .45
33-216 Cyprus × Ott. 770B	34			1	3	1	5	4	9	5	2	1	2	1								18.73± .41
33-217 Ott. 770B × Buda	58									2	3	5	14	9	8	5	2	4	4	2		24.17± .38
33-218 Buda × Ott. 770B	35												2	1	5	2	5	7	7	2		27.51± .28

0.316 respectively. While the small number of lines in each class makes it difficult to draw any definite conclusions on this character, these data do not follow those of Myers (18). In a cross of Ottawa 770B with Redwing he has reported evidence of a partial dominance of large seed size.

Height of Plant

The Cyprus variety is a short-strawed type. In studying the cross with Ottawa 770B, a medium tall variety, a comparison of height of F_2 plants with the mean height of their F_3 progenies showed a correlation of 0.354, with the 1% level at 0.325. This would seem to indicate that height of plant can be explained on a genetical basis. The distribution of the F_3 lines with their parents, grown in the season of 1935, is shown in Table VIII. The variability of the hybrids is greater than that of the parents, showing that segregation may have taken place. The fact that many plants are in lower classes than the parents and also that the mean height is less than that of the shorter parent would suggest a dominance of short-strawed plants. This is especially true when we consider that many of the segregates are in the heterozygous condition and might still be expected to show hybrid vigor.

Association of Characters

Relation of Iodine Number to Oil Content

The tendency of varieties producing large quantities of oil to produce oil of poor quality has already been indicated. Studies of partial correlation indicate that these characters are not correlated. The oil content of the parents did not differ to a very great extent. The correlation of oil content and iodine number within the different lines of the Cyprus parent was −0.388, of the Ottawa 770B parent 0.334, and of the Buda parent 0.314; with the 5% level at 0.444, 0.396, and 0.388 respectively. This agrees with Ermakov (6) who found no association of these characters among the different lines within a variety.

The correlation between oil content and iodine number in the progeny of Cyprus × Ottawa 770B was 0.163 and 0.141 where a correlation of 0.325 or higher is required to show significance. In this instance iodine number may have been inherited independently of the percentage of oil in the seed. Cole *et al.* (3) found that selection for quality in the oil of the soybean did not tend to depress the quantity present.

Relation of Color of Seed to Quantity and Quality of Oil

From the F_3 generation the nature of the selection in the F_2 was determined, *i.e.*, whether they were true breeding brown or yellow or were heterozygous for color of seed. Iodine numbers of these lines are summarized in Table IX.

The significance of the difference of the yellow compared with the heterozygous and the brown classes was calculated for each group of hybrids. In comparison with the P value of 0.01, it will be seen that these differences in the means of these groups are significant, especially in the cross Ottawa 770B × Buda. There was a tendency for a higher mean iodine number to be associated in each cross with yellow seed.

TABLE IX

IODINE NUMBER OF YELLOW-SEEDED LINES COMPARED WITH BROWN AND HETEROZYGOUS LINES IN THE F_3 GENERATION OF OTTAWA 770B × CYPRUS AND OTTAWA 770B × BUDA

Seed color	No. of lines	Mean iodine number	S.E.	*t*. value	P = 0.01
33-215 Ottawa 770B × Cyprus					
Yellow	10	183.7	5.21		
Heterozygous	15	177.8	5.62	2.64	2.807
Brown	10	178.2	3.15	2.86	2.878
33-216 Cyprus × Ottawa 770B					
Yellow	11	182.3	4.06		
Heterozygous	16	174.9	4.53	4.28	2.787
Brown	7	177.9	7.52	1.63	2.921
33-217 Ottawa 770B × Buda					
Yellow	27	194.2	2.77		
Heterozygous	21	187.6	2.79	8.16	2.575
Brown	10	188.4	2.35	5.87	2.575

Relation of Color of Oil to Quantity and Quality of Oil

From a practical standpoint, oil of high iodine value, low in pigment content, is desirable. However, in the hybrid progenies studied, neither the quality of the oil nor the oil content of the seed appears to be associated in any way with pigment content. The correlation between carotene pigment, measured in parts per million, and oil content was -0.204 while with iodine number it was 0.035. Both correlation coefficients are below the 5% level for significance.

Relation of Size of Seed to Quantity and Quality of Oil

Several investigators (1, 4, 13) have pointed out an association between seed size and percentage of oil, emphasizing the importance of seed size as an aid in selection for high oil content. It has already been shown in a survey of varieties that large-seeded varieties tend to be higher in oil content. Ermakov (6) has been unable to show a similar association when large- and small-seeded types within the same variety are analyzed. In this study the oil content and iodine number have been considered from lines of the parent and hybrids grown under the same environmental conditions. As shown in Table X, neither the parents nor the hybrids show any tendency for size of seed to be related to oil content or iodine number. The lack of association between these variables should facilitate the possible combination of these desirable characters.

TABLE X

RELATION OF SIZE OF SEED TO OIL CONTENT AND IODINE NUMBER. OIL CONTENT IN PER CENT *(o)*; IODINE NUMBER *(i)*; WEIGHT PER THOUSAND KERNELS *(m)*

——	r_{mo}	r_{mi}	5% level
Buda	−0.29	−0.35	0.39
Cyprus	− .19	− .27	.44
Ottawa 770B	.15	.19	.39
33-215 Ott. 770B × Cyprus	.16	.04	.35
33-216 Cyprus × Ott. 770B	− .19	.16	.35

Relation of Height of Plant to Quantity and Quality of Oil

In studying the relation of height of plant it was found that oil content was negatively and iodine number positively associated with height. The segregation of factors for height has already been indicated. In the reciprocal crosses of Cyprus and Ottawa 770B, correlations of −0.293 and 0.001 have been obtained between height and iodine number. In the cross Buda × Ottawa 770B the correlation was −0.347. Since a correlation of 0.349 or higher is required to show significance, no relation between height of plant and iodine number is indicated.

Discussion

The purpose of this project is to study the relation of quality and quantity of oil to other plant characters in flax and to determine their mode of inheritance. A highly significant positive correlation for high quality and quantity of oil would greatly facilitate selection for this combination. The results from the present survey of varieties are in agreement with those of Johnson (14) in indicating that the simple correlation is negative and significant while the partial correlation is not. Added information was obtained from the hybrid progenies of Cyprus × Ottawa 770B where segregation has been shown to have taken place. However, no association of these characters has been indicated by a study of either the parent lines or those of the hybrids.

Iodine number appears to be affected by several factors, with no suggestion of dominance of high or low quality. This does not agree with the data of Arny, as presented by Dillman (5), who suggests low iodine number to be dominant, and in backcrosses with parents of medium or high iodine number, a ratio of approximately 1 : 1 was obtained. The Bison variety, used by Arny as the parent for low iodine number, is considerably higher than Cyprus and may account for this difference.

Previous investigators (5, 14) have considered it important to select strains of flax with medium-large seed in order to obtain a satisfactory yield of oil. Since no significant relations were obtained between either oil content, iodine number or size of seed in the hybrids studied, greater progress in improvement of the flax crop may be expected from analyses of the hybrid selections. The refractometric methods have been found very practical for such determinations.

There are many agronomic advantages in large-seeded varieties aside from the possibilities of increased oil production. The distribution of seed size is of interest here, since it does not appear to follow that described by Myers (18), who also used Ottawa 770B as one of the parental types. Myers' data suggest a partial dominance of larger seed, whereas, in the distribution shown, in only one instance has the large parental size been regained. A dominance of small size would be in accordance with the recent theory of Fisher (9) that the wild species was evolved by the accumulation of dominant genes. Vavilov (23) has suggested that the wild progenitor of our common flax is the species known as *Linum angustifolium* in which the seed averages 1.50 gm. per thousand kernels. Vavilov believes that domestication is the result of the selection of recessive genes.

The few varieties of yellow-seeded flax that have heretofore been grown in Canada have been observed to yield oil of very high iodine value. Data from the segregation of these two characters indicate a very definite association of high iodine number with yellow-seeded segregates. Recently, Dillman (5) has published data of Arny which show an apparent linkage of yellow seed with high or medium iodine number. In a study of flower and seed type it has been shown both here and in other investigations (18, 21) that the inheritance of the white petal, involute flower type with yellow anthers and greenish yellow seed can be explained by the action of a basal factor or group of factors very closely linked. The morphological effects of this factor or group of factors might now be extended to include the physiological effect of quality in the composition of the oil.

Yellow-seeded varieties appear generally to be considerably lower in oil pigmentation than brown-seeded varieties. This may be of particular interest to the manufacturer of lightly colored paints and enamels. Morrell and Wood (17) point out that it is most difficult to obtain a bright pale oil that will give satisfaction in drying properties. Sometimes one of these properties is sacrificed at the expense of the other. The color specification for a refined linseed oil is not darker than 0.1 gm. potassium chromate dissolved in 100 gm. of sulphuric acid of a specific gravity of 1.84. In studying pigment content in a preliminary manner with the cross of Ottawa 770B × Buda there are indications that "carotene" pigment content may be dependent on several factors for its expression. The color of the oil was not correlated with either oil content or iodine number. The fact that yellow-seeded segregates with very high pigment content were obtained would indicate that oil color may also be independent of the color of the seed.

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CHEMICAL WEED KILLERS

II. FACTORS AFFECTING ESTIMATION OF TOXICITY OF LEAF SPRAYS[1]

By W. H. Cook[2]

Abstract

Chemicals applied to annual weeds frequently gave dosage-mortality curves that were not of the usual sigmoid shape. These departures appear to be attributable to the method of application, as the spray is only partly retained by the leaves and stems. The proportion of the applied quantity of poisonous constituent retained by the plant decreases as the volume of spray is increased, and increases as the concentration of the spray solution is increased. Leaf sprays will therefore be most effective if the minimum volume of solution required for coverage is used, and the effective dosage of the chemical obtained by adjusting the concentration.

A few indices sometimes used for estimating the efficacy of herbicides were compared with the mortality criterion. The number of leaves left, and the height of the living plants after treatment are of little or no value for estimating the effect of the chemical. The weight per unit area of the living plants remaining after treatment may be of some value.

Introduction

A review of the literature (4) shows that there is a great divergence between the dosages of a given chemical reported by different investigators to kill a certain species. In most cases this divergence is so great that it has been impossible to reach a definite conclusion concerning the relative toxicity of different chemicals. This variability appears to be attributable to three main causes:

1. The variation in the susceptibility of plants of the same species. It appears that most investigators studying chemical herbicides have failed to appreciate the magnitude of the variability that is encountered when apparently similar organisms are used. Added to this variation between individuals, is the variability contributed by the different soil and climatic conditions under which different experiments are carried out.

2. Drainage to the soil of a variable proportion of the applied spray. Considerable variability can be expected from this source since the relative proportion and efficacy of the poison applied to the aerial parts of the plant and to the soil are unknown.

3. The various criteria of effectiveness used by different investigators. Some experimenters report the percentage mortality resulting from a given treatment, some report the weight, height or other measure of growth, others the growth rate, yield of economic crop following treatment, etc. Although

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[2] *Biologist, National Research Laboratories, Ottawa.*

the mortality criterion is the one most commonly used in precise studies of toxicity, and is the one that has been studied most from the standpoint of suitable methods for estimating the effective dosage and its variability, it cannot always be employed in herbicide studies under field conditions. It is possible, however, to gain some idea of the relation between mortality and the other criteria, so that the significance of the results can be estimated.

It is evident that little can be done toward reducing the variability arising from the different susceptibility of individual plants of one species, but the error arising from this source can be decreased by adequate replication. The form of the dosage-mortality curve is determined by the variation in susceptibility, and if the usual sigmoid curve is obtained, methods such as those of Trevan (5) and Bliss (1, 2) can be applied to estimate the effective dosage, and to evaluate the extent to which these estimates may be in error. If the dosage-mortality curve is not sigmoid, consideration must be given to the possibility of other systematic errors affecting the results. A few of these are discussed in this paper.

Most of the experimental data presented were obtained in connection with an investigation into the relative toxicity of different chemicals to annual weeds. As the materials and methods employed have been reported in an earlier paper (3) they need not be described here. It is important to note, however, that the chemicals were usually applied as a 10% solution, and the dosage was varied by adjusting the volume of solution sprayed on the plants.

The author regrets that he had no opportunity to determine whether the hypotheses presented are applicable under field conditions. Nevertheless, they are presented here in the hope that they may be useful.

The Dosage-Mortality Curve

In most cases where a toxic substance has been administered directly into the organisms, or where the latter can be subjected to a known concentration of the poison, as in fumigation, a sigmoid dosage-mortality curve has been obtained. This represents the integrated frequency curve descriptive of the variable susceptibility of the individuals tested. The exact position, shape and slope of this curve usually differs somewhat for each poison and species under investigation, but in general the greatest slope is in the region of 50% mortality, and comparative tests of different substances can generally be made most accurately in this region. During the course of the investigation already mentioned (3) an attempt was made to determine the shape of the dosage-mortality curve obtained with certain species and chemicals. It was found that these curves often departed considerably from the usual sigmoid form, and in consequence the certainly lethal dose, rather than the median lethal dose, was used as the measure of toxicity and, with two exceptions, no attempt was made to determine the dosage-mortality curves for all chemicals and species. Owing to the number of dosages employed in certain instances, however, sufficient data were collected to permit the nature of the curves to be approximately determined. These curves are shown in Fig. 1.

Chart A in the upper left-hand corner shows the curves obtained from the application of formic acid and zinc sulphate to stinkweed, *Thlaspi arvense* L., and formamide and sodium acetate to wild mustard, *Brassica arvensis* (L.) Ktze. These two weeds have about the same resistance to toxic sprays, and were the most susceptible annuals tested (3). All of these curves are approximately sigmoid with the exception of that for the least toxic substance, sodium acetate.

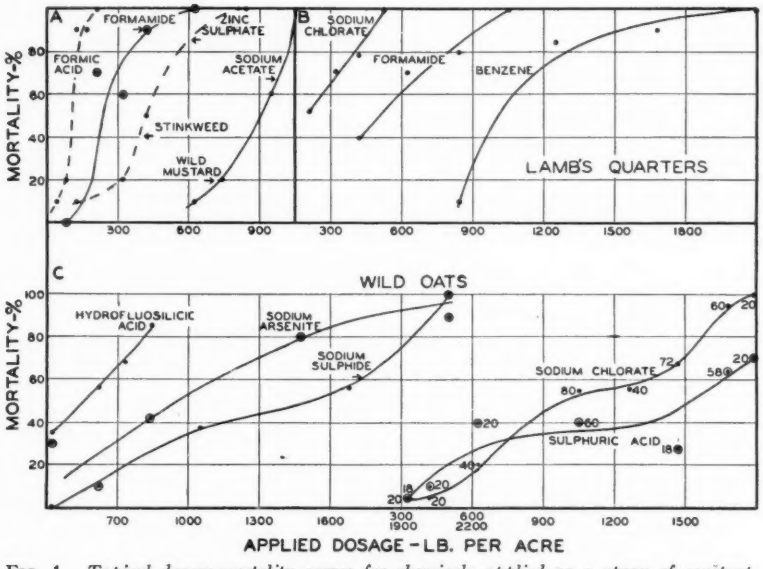

FIG. 1. *Typical dosage-mortality curves for chemicals applied as a spray of constant concentration.*

Chart B (Fig. 1) shows the effect of three chemicals on lamb's quarters, *Chenopodium album*, L., which is approximately twice as resistant to chemicals as stinkweed and wild mustard. The two more toxic materials show a practically linear relation between 50 and 100% mortality. On the other hand the curve for benzene rises steeply at first, but gradually decreases in slope as it approaches 100% mortality.

Chart C (Fig. 1) represents curves obtained with wild oats, *A. fatua* L., which is approximately seven times as resistant as stinkweed and wild mustard, judging from the certainly lethal dose applied as a spray. On the left-hand side the approximate nature of the curves for hydrofluosilicic acid, sodium arsenite and sodium sulphide has been drawn from the few data available. Of these, hydrofluosilicic acid and sodium arsenite show an approximately linear relation between dosage and mortality. Sodium sulphide, on the other hand, shows a slight tendency to flatten out in the region of 50% mortality. The two curves in the lower right-hand corner represent the data obtained from spraying with sodium chlorate and sulphuric acid. Many

more data were available for plotting these two curves, and at least their shape can be fixed with some degree of certainty. The number appearing beside each point represents the number of individual plants used for its determination. It is obvious that they are not sigmoid, both tending to flatten out in the region of 50% mortality.

The main point illustrated by Fig. 1 is that a sigmoid relation between dosage and mortality was obtained when the more toxic chemicals were applied to the more susceptible species of plants. As the resistance of the species under test increases, or the toxicity of the chemical decreases, this relation disappears and the curves assume various shapes, but finally tend to flatten out at a certain mortality, and rise again at high dosages. The shape of these curves could be attributed to a decided asymmetry in the distribution of the susceptibility of the individual plants, but it seems more likely that the first portion of the curve results from the action of the spray on the leaves, while the portion of the curve beyond the flat part represents the additional toxic effect of the chemical in the soil. This leads to a consideration of the second source of variability, namely, the method of application.

Relative Amounts and Efficacies of Spray Retained by Foliage and Entering Soil

The application of a toxic solution to growing plants as a spray results in a certain proportion being retained on the plant, while the rest drains to the soil. The proportion of the spray remaining on the plant may be termed the "retained" dosage and will be affected by many factors such as the size of the plant, density of growth, type of leaf and stem, the retentive properties of the plant surfaces for the toxic solutions, etc., but for the most part it will be determined by the volume of spray required to carry the effective dosage of the poison. The remainder of the applied dosage will drain to the soil where it may be detoxicated, or exert an additional toxic effect. This brings up the question of the relative efficacy of chemicals applied directly to the foliage, or indirectly through the soil. Doubtless this varies greatly with different chemicals and soils. It seems likely, however, that most of the leaf sprays, or contact poisons, act mainly on the foliage when applied in the dosages ordinarily used, but it must be recognized that most of these substances could potentially exert a toxic action through the soil if applied in sufficient quantity. Many such substances can be detoxicated in the soil by neutralization, adsorption, conversion to insoluble compounds, etc., while dilution in the soil solution affects all poisons, and could easily reduce the concentration of small dosages below the minimum required to be effective. These considerations indicate that the dosage required to produce a certain mortality through the soil would generally be higher than that required on the foliage. Under these conditions, the part of the solution draining to the soil will be lost if small volumes are applied, but may produce an additional toxic effect if large quantities are used. Such effects could easily modify the shape of the dosage-mortality curve. Certain chemicals may act through the soil only, but the consideration of these is beyond the scope of this paper.

It is evident that some method of estimating the "retained" dosage from the "applied" dosage of leaf sprays is required. The maximum amount of spray which plants of a given species, of size A (in terms of surface area of plant per unit area of land), will retain is unknown, and there are no data available from which it could be estimated, but it can be designated as V_m gallons per acre. Now let it be assumed that:—

(1) The density of plant growth is such that the entire surface presented to the spray is plant leaves or stems, so that none of the spray falls directly on the soil, but reaches it only be drainage from the plants.

(2) All portions of the surface area of the plant are equally accessible to the spray.

(3) Any excess of liquid on any portion of the plant surface will drain off directly and not wet an otherwise dry area.

The last two assumptions are not strictly justifiable but they are sufficiently correct for purposes of estimation, and since they act in opposite directions, the resulting inaccuracies will tend to cancel each other.

Since an increment of spray may fall with equal probability on any portion of the entire surface area of the plant, it follows that the amount retained will be that held by a constant proportion of the dry area remaining at the time of application, while the dry area will decrease continuously as the applied volume increases. The amount retained out of each succeeding applied increment will therefore diminish continuously. The relation between retained and applied volumes can therefore be represented by the general equation for continuous depreciation of the type:

$$dR = e^{-V}dV,$$

where dR is the quantity retained when an increment dV of solution is sprayed on after V gallons per acre have been applied. In this equation all constants expressing the retentive abilities of the system under consideration are unknown and have been taken as 1, so that only the shape of the retention curve, and not its position and slope, are described by the equation.

Integrating the above equation gives

$$R = 1 - e^{-V}$$

where R is the amount retained after V gallons have been applied, while the proportion of the total applied spray retained is

$$\frac{R}{V} = \frac{1 - e^{-V}}{V}.$$

In all cases the proportion of the spray draining to the soil can be obtained by difference.

The retention curves can be deduced from the above equations by plotting the proportion retained, for different values of V, against V_m as defined earlier. These curves appear in B, Fig. 2, and it can be seen from the retention curve (R) that when a quantity of spray equal to V_m, the maximum amount

that can be retained, has been applied, only about 63% of it remains on the plant. When a quantity equal to $3V_m$ has been applied the portion retained is about $0.95\ V_m$, or about one-third of the spray employed. If still larger volumes are applied most of the spray in excess of $3V_m$ drains to the soil.

Since the proportion of the spray draining to the soil will be ineffective as a leaf spray, the retained dosage rather than the applied dosage should be employed for constructing dosage-mortality curves for substances acting only on the leaves. In A, Fig. 2, two symmetrical distributions of the susceptibility

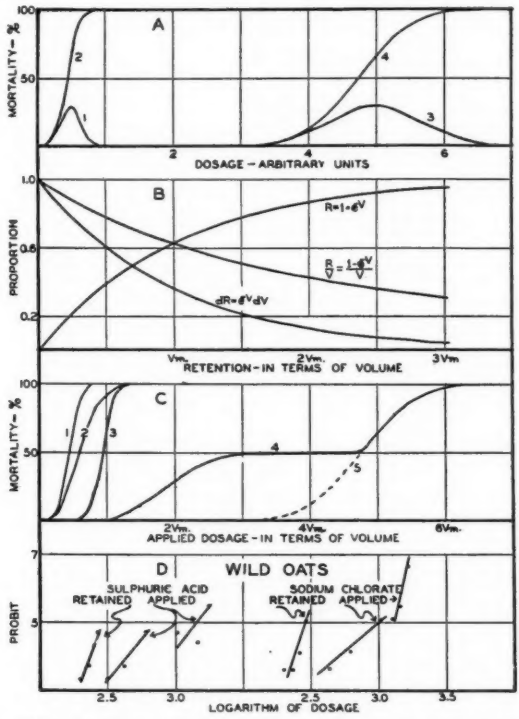

FIG. 2. *Effect of partial retention on the form of the dosage-mortality curve.*

of individual organisms to a hypothetical poison have been constructed (Curves 1 and 3), together with the integrated frequency, or sigmoid dosage-mortality curves obtained from these distributions (Curves 2 and 4). In both cases it is assumed that the entire dosage was retained by the organism, or administered into it, so that the distribution is not affected by the difference between the "applied" and "retained" quantities. The left-hand distribution and mortality curves apply to a relatively toxic material having a median lethal dose in the region of 0.5 dosage units. The right-hand curves apply to a less toxic substance having a median lethal dose in the region of five dosage

units. The variation in the susceptibility has been assumed to be proportional to the decreased toxicity, which results in the distribution shown in the graph. Although these examples may be somewhat exaggerated, Curves 2 and 4 are analogous to what might be expected if a typical leaf spray were applied to the aerial parts of the plant, and to the soil, respectively.

In C, (Fig. 2) the mortality resulting from the application of hypothetical substances is plotted against the applied dosage in terms of V_m. Curve 1 is a reproduction of Curve 2 in A, and would be the type obtained if all of the toxic spray was retained. By computing the proportion of the spray retained by the plant from the curves in B, and assuming that only this portion of the spray was effective, the dosage-mortality curve is changed in slope and position to that represented by Curve 2. This changed position suggests a new distribution of the susceptibilities, but it is, in fact, due entirely to the difference between the applied and retained dosages. Since the discrepancy between the retained and applied dosages increases as the volume of spray is increased, the divergence between the "observed" and "real" dosage-mortality curves will increase as the toxicity per unit volume of the solution decreases. Since the plant can retain only the volume V_m gallons of spray per acre, it is obvious that if this volume does not contain sufficient chemical to kill even the most susceptible plants, no mortality will result until the material is added in sufficient quantity to act through the soil. If, however, the quantity V_m carries sufficient poison to kill a certain percentage of the more susceptible individuals, this percentage mortality will be approached asymptotically, and will be reached, for all practical purposes, when about $3V_m$ gallons have been applied, since this corresponds to a retained quantity of approximately $0.95\ V_m$. This behavior is illustrated in Curves 3 and 4 in C, Fig. 2. Curve 3 depicts the same distribution as Curve 1, but has been moved to the right, so that the median lethal dose corresponds to the amount contained in V_m gallons of spray, and is again obtained by assuming that all of the spray is retained by the plant. Since only part of the applied dosage is retained, the mortality, plotted against the applied dosage, will be represented by Curve 4, and will reach a constant mortality in the region of $3V_m$ applied dosage. Obviously, different chemicals, or solutions varying in toxicity per unit volume, can produce dosage-mortality curves which reach constant mortality values anywhere between 0 and 100% mortality.

Although certain toxic sprays may produce only a partial mortality from their action on the leaves and stems, owing to the limited retaining power of these parts, it is also possible that some may show an increased mortality at extremely high dosages from their action through the soil. In order to illustrate the effect of the combined action on the shape of the dosage-mortality curve, Curve 4 in A has been assumed to represent the dosage-mortality relation when the chemical acts through the soil only, and has been reproduced as Curve 5 in C. The flat portion of Curve 4 in C, representing the partial mortality at the maximum retained dosage, intersects this curve, and if the substance in question can act through the soil, the complete dosage-mortality

curve will, as a limiting condition, follow the solid line representing the combination of Curves 4 and 5. Any interaction of the two toxic effects will alter the shape of this combined curve near the point of intersection, but no data are available on this point. The point of intersection of the two curves will naturally depend on the mortality produced from the action of the chemical on the leaves, the effective dosage in the soil, and any tendency for the action at the two loci to be additive. The general similarity in shape between the solid line formed by combining Curves 4 and 5 in C, and the curves obtained experimentally with sodium chlorate and sulphuric acid applied to wild oats (Fig. 1), supports the several hypotheses here deduced from theoretical considerations.

A further study of the dosage-mortality curves was made by plotting the probit, or probability unit, against the logarithm of the applied dosage. These conversions have been used by Bliss (1) to convert sigmoid dosage-mortality curves to straight lines suitable for precise statistical treatment. The probit is essentially the number of standard deviations describing the distribution of the variable susceptibilities, chosen in such a manner as to avoid negative values. The logarithm of the dosage, rather than the dosage itself, has been used, since the majority of the experimental data so treated have been found to yield a straight line, and the inference is that the susceptibilities are distributed as a logarithmic, rather than as an arithmetical, function of the dosage. When this double conversion was applied to the sigmoid curves in Fig. 1, the resulting curves were slightly concave upwards, regardless of whether "applied" or "retained" dosages were considered, but as these curves were based on rather few data, the applicability of the double conversion to herbicide tests must await the results of further experiments. When the double conversion was made with the dosage-mortality curves based on applied dosages of sodium chlorate and sulphuric acid (Fig. 1), the results obtained with both chemicals could be fitted graphically by two straight lines, which appear in D, Fig. 2, and support the contention that the original curves are the result of two separate effects, *i.e.*, one on the aerial parts of the plant, and the other through the soil.

The straight line representing the first part of the dosage-mortality curve, and attributed to the action of the chemical on the leaves, was converted to the retained dosage, using the curves in B, Fig. 2, and assuming V_m to be 300 gallons per acre. In order to arrive at this value of V_m, the dosage at the beginning of the flat part of the curves in Fig. 1 was taken as $3V_m$. This gave V_m as 220 and 350 gal. per acre for sulphuric acid and sodium chlorate respectively. Some preliminary direct measurements made on similar plants after spraying 1000 gal. per acre indicated that the retained quantity lay between 120 and 320 gal. per acre. The curves plotted from the "retained" quantities are altered in position, and have a considerably greater slope. Extrapolation of these lines indicates that the C.L.D. to wild oats of both sulphuric acid and sodium chlorate when applied to the leaves only is about 850 lb. per acre. Little significance can be attached to this value until V_m, and

other properties of the chemicals under investigation, are known more accurately. For example, sodium chlorate is generally believed to act almost entirely through the soil, and it is also known to act slowly compared with sulphuric acid. Both of these factors may affect the shape of the dosage-mortality curve, and may account for the fact that 500 lb. per acre of sodium chlorate applied to weeds was found to kill wheat planted in the soil shortly afterwards (3), whereas the curve in Fig. I shows only a 55% mortality of wild oats following applications of 1000 lb. per acre. On the other hand, this difference could also be explained by a difference in the susceptibility of the two species of plants.

The foregoing considerations show that the loss of toxic solution from the foliage must affect the efficacy of herbicides. If the chemical acts through the soil only, the concentration of the solution is of secondary importance, but if it is used as a leaf spray the concentration must be such that a lethal dosage of the substance will be retained by the plant. Furthermore, since the proportion of spray reaching the soil increases with the volume of spray used, this loss will be reduced by using as concentrated a solution as possible. On the other hand, a sufficient volume of spray must be applied to give at least the minimum coverage required to produce complete mortality. The best concentration to use in practice will therefore be one containing a certainly lethal quantity of the chemical dissolved in a volume of solution somewhat in excess of that required to give the minimum effective coverage. It is therefore necessary to determine experimentally the volume of solution required for effective coverage and the maximum retained volume, for a given size of plant of the species in question, before the C.L.D. can be determined by varying the concentration. In this connection, the size of the plant is extremely important, and in the past has not received the consideration it merits. Its estimation by cutting and weighing the plants from small areas selected at random would permit some standardization of the lethal dosage and volume required, as functions of plant size rather than per unit area. Since the chemical must affect a certain proportion of the cells, or constituents of the plant, it seems reasonable to believe that the lethal dosage will change in proportion to the weight of the plant. The capacity of the plant for retaining the toxic spray will vary as the area of the plant surfaces changes, but since the major part of the growth of most weeds is in two dimensions, it seems likely that the area and weight will be closely related. Hence when the lethal volume and concentration of spray for a given size of plant are known, the dosage required for other sizes of plants will be obtained sufficiently accurately if the volume of solution only is changed proportionately.

Although concentrated solutions might result in retention of a greater proportion of the toxic constituent, it is possible that the absorption of the poison by the leaves may be the factor limiting the efficacy of certain substances. Where this is the case, concentrated solutions might be of little value, but further investigation is required before a definite statement can be made.

Criteria of Toxicity

The use of different criteria by different investigators (4) renders many of the published results non-comparable since the relation between the quantities measured and the mortality are unknown. For instance, a reduction of 50% in the weight of plants per unit area might not result in the death of any of the plants.

In the first paper of this series (3), reporting work in which the certainly lethal dose was used as an index of the relative toxicity of different substances, the results of a considerable number of dosages leading only to partial mortality were omitted. In these tests the green weight of the plants, their height and the number of leaves per plant, were observed and expressed as a percentage of the same quantities determined on the untreated control plants grown at the same time under similar conditions. The relation between these quantities and the observed mortality for each of the four kinds of plants studied, are shown in a series of scatter diagrams in Fig. 3. Each point

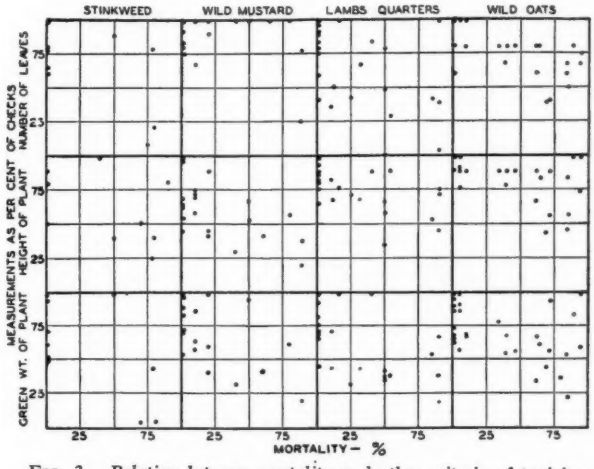

FIG. 3. *Relation between mortality and other criteria of toxicity.*

represents the results for a given dosage of a given chemical. All the chemicals are grouped together in the appropriate diagram; no attempt has been made to distinguish between them since any reliable measurement of the efficacy of the poison must apply to all substances if it is to be generally useful. Two or three of the points on some of the diagrams represent the effect of one substance applied at different dosages.

Accepting mortality as the standard criterion of toxicity, it is evident that the suitability of any new criterion will depend on the degree to which it is correlated with mortality, and also on the constancy of this relationship for different species of plants. Examination of Fig. 3 shows that while there is some agreement between the various criteria and mortality for some of the

species, the variability is so great that the degree of correlation would never be high. With respect to the constancy of the relations between different species, it appears that each of the weeds differs somewhat, but from this standpoint the value of the criteria perhaps increases in the order: number of leaves, height of plant, weight of plant. When the high variability between the various criteria and mortality, and between different species, are both considered, it appears that the weight index is the only one that might be useful as a measure of toxicity.

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THE PROTECTIVE LAYERS OF THE APPLE[1]

By Hugh P. Bell[2]

Abstract

The development of the four protective layers of the apple, namely the hairs, cuticle, epidermis and hypodermis, is described in detail, from the time the flower emerges from the winter bud early in May, until harvest time early in October. The gradual loss of living and active cell contents is recorded for each layer. Measurements of cuticle thickness for the period of the study are listed and an approximate date is given for each stage of development. The descriptions of tissue morphology are supplemented by ten illustrations.

Introduction

The structure and development of the protective layers of the apple are fairly well known, but there is a great variation from locality to locality in the time of the year when each stage in this development is reached. The condition of these protective layers at any one time has an important bearing on the reaction of the young fruit to sprays and weather conditions to which it may be exposed at that season, hence, for each locality, it is desirable to know the approximate date when each protective tissue is developed. The study outlined below was undertaken at the request of the Pathologist-in-Charge at the Laboratory of Plant Pathology, Kentville, N.S., to determine the rate and time of development in Nova Scotia.

The apple studied was the McIntosh Red. The material was collected during the summer of 1934, by the staff of the Laboratory of Plant Pathology, Kentville, N.S. It was killed in chrom-acetic, and imbedded in paraffin as described by Bell and Facey (1). The stains used were acid fuchsin for cell wall, soudan IV for cuticle, and iron alum haematoxylin for cell contents. During the summer of 1934, the trees from which the material was collected were in full bloom on June 2, and the fruit was ripe and harvested by the end of September or early in October.

The development of the protective layers could be traced from the first appearance of the flower primordia, but there is no practical interest in these layers while they are still completely enclosed in and covered by the scales of the winter bud. Interest in these layers starts when the surface of the young apple is first exposed to weather conditions and to early sprays. Hence the earliest condition described in this study is that prevailing just at the time the young flowers start to emerge from the winter buds. In the spring of 1934, when the collections for this study were made, this stage was reached during the second week of May, that is about three weeks before full bloom.

The outer protective region of the apple includes four layers of tissue. These are: the coating of epidermal hairs, the cuticle, the epidermis and the hypodermis. The development of each layer will be described by itself and

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Contribution from the Botanical Laboratories of Dalhousie University, Halifax, Nova Scotia, Canada.
[2] *Professor of Botany, Dalhousie University.*

then, to give an idea of the structure of the protective region as a unit at significant periods of the growing season, the information will be presented in tabular form. The findings recorded below agree in general with those of Tetley (4, 5), who reported on the development of the protective layers for a number of varieties of apple commonly grown in England. A few minor disagreements were noticed regarding certain details. These will be mentioned as each structure is discussed.

In layers that are cellular in structure, the type of reaction to changes in growth and environment depends largely on the condition of the protoplasmic cell contents. For instance, active cells can repair minor ruptures or injuries by the growth of new cells, but these active cells are very sensitive to an unfavorable environment of either weather or sprays. Conversely thick-walled semi-living cells cannot as a rule repair a rupture in their layer, but they are highly resistant and are not easily injured by an unfavorable environment. Hence while these morphological changes are taking place, it is important to know the extent to which the cells retain their vital properties, and an attempt has been made to record this information for each protective layer and for each stage of development. The protoplasmic contents were used to indicate the condition of the vital activity of the cell. Where cell division could be demonstrated, the cells were regarded as active. Where the nucleus and other protoplasmic contents had completely disintegrated, the cells were judged to be dead or at the most semi-living. All stages between these two extremes were observed.

The Development of Each of the Four Protective Layers

The Coating of Hairs

At the time the young flowers start to emerge from the winter buds, the ovary is covered with a dense mat of tangled hairs. Each hair is a modified epidermal cell and is living and active. The proximal portion of a typical hair as it exists at this time is shown in Fig. 1. The surface of the hairs is such that it is not easily wetted, and in addition this layer encloses myriads of minute and more or less enclosed air spaces. Consequently this mat of hairs is a very efficient protection to the young ovary. As the ovary enlarges, the epidermal cells between the hair bases multiply by cell division. As a result the hairs become more widely separated. Another important change takes place during the three weeks immediately preceding full bloom. The layer of cuticle that is being laid down over the outer surface of all the epidermal cells is comparatively much thicker just where the hair base joins the outer tangential wall of adjacent epidermal cells, and this comparatively thick layer of cuticle extends into the hair base and down the inner surface of the radial walls till it reaches the inner tangential wall at the base of the cell. This is illustrated in Fig. 4. Thus, at the time of full bloom, although the ovary is still densely tomentose, the cuticle has already started to replace the hairs as the outside protective layer. Also as the hairs are now practically cut off from other living cells, they are not so active or efficient as they were

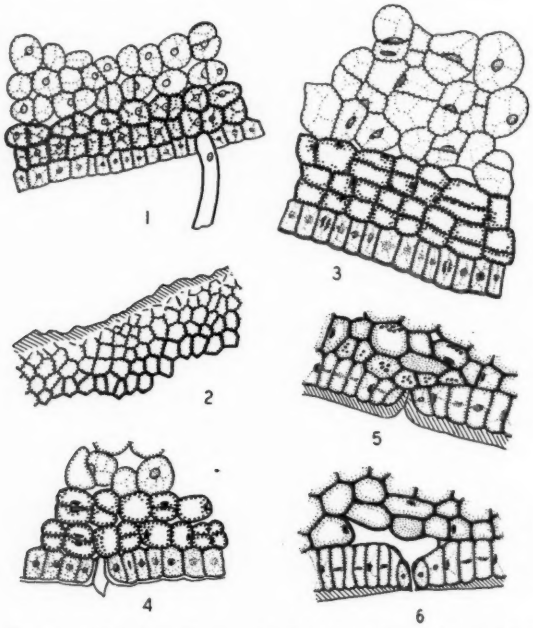
at first. This is indicated by the fact that they are gradually becoming more brittle and more easily wetted. The deposition of cuticle continues and by the middle of June the space formerly occupied by the hair base is completely closed. This closing of the basal cell continues, until finally the location of the hair base cannot be identified with certainty. Apparently this formation of cuticle does not of itself cause a separation of the hair from the surface of the ovary, for the proximal portion of the hair may still be found attached even after the cuticle is quite thick, but by the middle of June the slightest touch will cause the hair to break across just at the outer surface of the cuticle. Tetley reports (4, pp. 163, 165, 286) that in the varieties she studied, a rupture in the protective layer frequently occurred at the base of the hair. This was not observed in the McIntosh material examined during this study but, as stated above, the cuticle always filled in the hair base so that the sub-epidermal layers were never exposed and the hair base was finally completely filled in and covered over by a smooth and continuous layer. During the latter part of June the hairs gradually disappear from all exposed parts of the apple, but they may still be found, well on into the summer, in the depressions at the calyx and stalk ends. By that time they are so widely separated and so insecurely attached, that they have ceased to afford any protection.

The Cuticle

At the time the young flowers start to emerge from the winter buds, the cuticle cannot be identified with certainty if one relies on a cross section only (Fig. 1), but it is present at that time, though it is not of uniform thickness. To see it, it is necessary to make a tangential section, then by using soudan IV, a distinct layer of cuticle will be seen (Fig. 2). This layer gradually thickens until by the time of full blossom it is from 1 to 1.7μ thick, and by using soudan IV it can be demonstrated in cross section (Fig. 3). However it is still not a uniform layer for it varies in thickness from spot to spot. This unevenness of the first cuticular deposit does not agree with the findings reported by Lee and Priestley (3, p. 527). They describe the first deposition of cuticle as, "either spreading evenly or thickening where it lies over the line of the middle lamella lying between two contiguous cells." In the McIntosh apple the first deposition of cuticle could be described as almost "patchy", with the thicker patches bearing no relation to the cell structure of the epidermis, and there was no evidence of thickenings over the ends of the radial walls. Also the cuticle at this stage is thicker at the bases of the hairs into which it extends (Fig. 4).

By the third week of June (Fig. 7) the hair bases are all filled in and the cuticle is continuous except over the stomata (Fig. 6) or young lenticels, where it shades off to nothing. It has an almost smooth outer surface, but its inner surface conforms to the irregularities of the epidermis. During August this inner surface of the cuticle starts to invade the epidermal layer in the form of V-shaped wedges between the epidermal cells; this continues till finally, at the time of harvest, the cuticle reaches the hypodermal layer, and may even extend under parts of the epidermis. Its final thickness on the ripe apple is

about 23μ, but owing to the irregularity of its inner surface it is impossible to express this cuticle thickness satisfactorily by one measurement only, for in addition to the inward extensions of the cuticle, the outer tangential walls of some epidermal cells are more convex and protruding than others. Above these protruding cells the cuticle may be very thin. This is not so characteristic of the very early stages, but from about June 8 on, three measurements



FIGS. 1–6. FIG. 1. *May 14. The proximal portion of an epidermal hair is included.* FIG. 2. *May 18. A tangential section.* FIG. 3. *June 1. In this drawing the unevenness in the thickness of the cuticle is not indicated.* FIG. 4. *June 1. A hair base.* FIG. 5. *June 15. A hair base filled by cuticle.* FIG. 6. *June 15. A stoma. All ×290. In Figs. 2, 5 and 6, the cuticle is singly cross hatched. With the exception of Fig. 2, all are radial sections.*

are necessary for a complete picture. These are: (i) the maximum thickness at points exactly opposite the ends of the radial walls between the epidermal cells; (ii) the minimum thickness at points opposite the centres of those epidermal cells which have protruding, convex, outer tangential walls; and (iii) the thickness opposite the centres of what appeared to be normal cells. This third figure is probably the nearest to what could be termed the "thickness of the cuticle". These measurements are given in Table I. Each figure is the average of a number of readings, but all the readings for any one measurement were taken from one specimen only. The information is given in this way to indicate the fact that in different specimens of the same date and even in different parts of the same fruit the thickness of the cuticle may vary

TABLE I

THICKNESS OF THE CUTICLE IN MICRONS

	Over the centre of cells with protruding convex outer walls	Over the centre of cells with nearly flat outer walls	Over the intersection between two epidermal cells
June 8	2.0	3.0	4.5
June 11	2.4	4.6	5.4
June 15	3.5	4.7	5.8
June 18	4.3	5.8	6.4
June 22	4.7	5.5	6.2
June 25	4.6	6.2	6.8
June 25	6.2	7.6	9.0
June 29	6.2	8.5	9.7
July 2	6.2	7.7	10.9
July 6	7.0	9.3	11.7
July 9	5.4	8.5	10.1
July 16	7.0	10.9	11.7
July 16	7.0	10.1	12.4
July 20	6.6	9.3	12.4
July 23	9.3	12.4	13.2
Aug. 3	8.5	11.7	14.0
Aug. 10	10.9	14.0	18.7
Aug. 17	12.4	16.3	19.5
Aug. 17	12.4	15.6	20.2
Aug. 24	9.3	15.6	20.2
Aug. 31	12.4	15.6	20.2
Sept. 7	11.7	15.6	20.2
Sept. 21	12.4	16.3	21.8
Sept. 29	18.7	23.4	25.7

May 18. Evident in tangential section, but too thin and uneven to measure accurately.
May 28. About 0.7, but uneven in thickness.
June 1. About 1.0, but uneven in thickness.
June 4. From 1.1 to 1.7, but uneven in thickness.

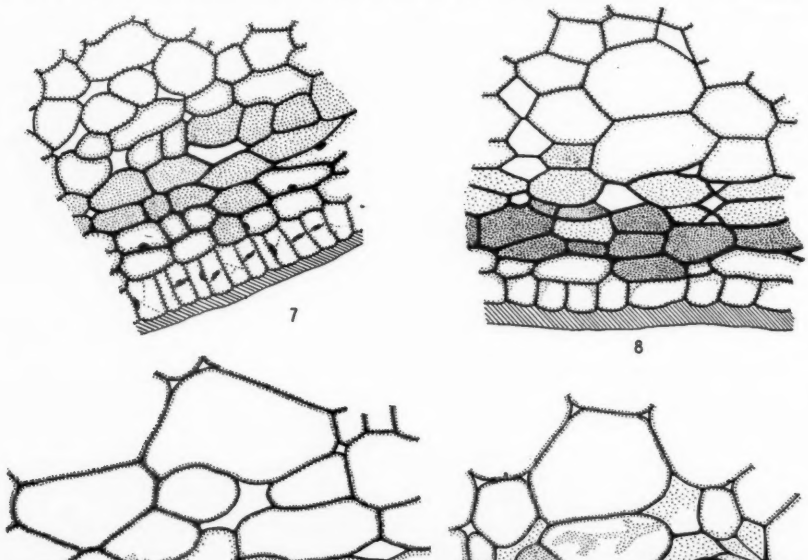
slightly. This is indicated by the figures given for June 25, July 16 and August 17. In general, however, the cuticle increases in thickness at a uniform rate from about the time of full blossom until the maximum thickness is reached about two weeks before harvest.

The Epidermis

At the time the young flowers start to emerge from the winter buds, the epidermis is a layer of active thin-walled isodiametric cells, measuring from 10 to 11μ both tangentially and radially. In a section at right angles to the surface, they are rectangular (Fig. 1), and in a tangential section they are round or roughly hexagonal in outline (Fig. 2). During the last three weeks of May, while the young flowers are emerging from the winter buds and the ovaries are starting to increase in diameter, the epidermal cells divide rapidly by radial walls, and mitotic figures may be seen in a number of cells in almost any section. This division by a radial plane is accompanied by a rapid increase in radial measurement, while the tangential measurement may be slightly reduced. The measurements at the time of full bloom are about 18μ radially, by about 9μ tangentially. The epidermis is then a closely packed layer of columnar cells and remains as such for two or three weeks.

The greatest radial measurement observed was 23μ in a specimen collected on June 15 (Fig. 5). During these first two or three weeks of June, cell division gradually ceases and the protoplasm becomes less dense, so that by the middle of June (Fig. 7), cell division is no longer observed and although both nucleus and cytoplasm are still distinct, the nucleus is often against the side wall and the cytoplasm is always vacuolated and chiefly peripheral. That is, the cells are still living, but they are becoming less active. There is also a slight increase in the tangential measurement of the cells. At this time (the middle of June) stomata, which appear to be structurally normal, are common throughout the epidermis (Fig. 6). The later condition of these stomata and the development of the lenticels were not followed in this study, but have been described in detail by Clements (2).

From the middle of June on, the cells of the epidermal layer change rapidly in regard to vital activity, cell contents and cell outline. Cell division could not be detected and all evidence would suggest that it does not again occur in this layer. Accommodation to the rapid increase in circumference appears



FIGS. 7–10. FIG. 7. *June 27.* FIG. 8. *July 16.* FIG. 9. *August 17.* FIG. 10. *September 29. All* ×290. *In all figures the cuticle is singly cross hatched.*

to be by means of cell enlargement in a tangential direction, so that by the middle of July the epidermal cells have changed from the radially elongated closely packed columnar type seen in Figs. 6 and 7 to the more plate-like tangentially elongated type seen in Fig. 8. At the same time the protoplasmic contents become less dense and extensively vacuolated and it becomes difficult to distinguish the nucleus. At this stage the cells vary greatly in size, but their tangential measurement may reach 32μ and in certain cells the radial measurement may be reduced to 14μ.

During the last part of July and the first part of August, these epidermal cells start to separate. This separation starts on the outer surface and the spaces between the epidermal cells become occupied by V-shaped invasions of cuticle. In places the epidermal cells appear to be grouped in pairs. The protoplasmic cell contents become irregular and disorganized (Fig. 9). During the last part of August and throughout September this process continues. The cells do not continue to elongate tangentially, but they do become more flattened radially. Some even collapse completely. They also become more widely separated, till by the end of September (Fig. 10) the cuticular invasion reaches the hypodermal cells and even extends under the inner wall between the epidermal and hypodermal cells. Also the protoplasmic contents have completely disintegrated and appear as dense or granular patches adhering to the cell wall.

The Hypodermis

At the time the young flowers start to emerge from the winter buds, the hypodermis consists chiefly of two layers of living and active cells (Fig. 1). The outer layer is continuous, but here and there the inner layer may not be differentiated. On the other hand in a few places the hypodermis may be three cells thick. These cells are similar in shape to the adjacent parenchyma cells of the cortex. The only difference is that the hypodermal cells have a thicker wall and their contents are more densely granular. In origin these hypodermal cells are primary tissue, and were originally part of the cortical parenchyma.

During the three weeks immediately preceding full bloom, the hypodermal cells multiply by an indefinite number of radial divisions. This enables the layer to accommodate itself to the increase in circumference without change in cell shape. At the same time each hypodermal cell divides once by a tangential wall. As a result of this tangential division, the average thickness of the hypodermis is increased to four layers, and this is the condition prevailing at the time of full blossom (Fig. 3). The origin of these layers is quite evident, for the daughter cells directly subtend each other in a radial direction, and are so definitely paired that the four rows have the appearance of two double rows. But it should be noted that the original slight variation in thickness is now more pronounced. For where there was originally only one layer there are now two (one double row), and where there were originally three layers there are now six (three double rows). Intercellular air spaces may occur at the corners of the cells (Fig. 4). These air spaces never become

filled with wall material as in typical collenchyma, but later disappear as the result of crushing. The cell walls have thickened slightly and in a section the layer is made conspicuous by the cell contents which are denser and more granular than in either the epidermal or cortical cells. The nucleus and cytoplasm are easily identified and appear to be quite normal. The cytoplasm is vacuolated and largely peripheral. The size of the hypodermal cells at this stage is from 12 to 18μ tangentially and from 9 to 12μ radially.

Very shortly after full bloom, the development of the mature features of the hypodermis starts. This development involves the following morphological changes. The cells become displaced from their original radial and tangential rows, and at the same time they become distorted, elongated tangentially, flattened radially and in many cases crushed. Air spaces appear and disappear. The cell wall is thickened. The protoplasmic cell contents become indistinguishable and the cells become filled with a dense substance which may be continuous and coarsely granular or discontinuous and globular (Fig. 5). This differentiation of the hypodermis is a continuous process and cannot be divided into periods. Figs. 7 to 10 and the detailed description given below will indicate the rate at which this development takes place during the summer.

By the third week in June (Fig. 7) the enlargement of the ovary has resulted in many of the hypodermal cells becoming displaced. The original radial arrangement of the cells no longer exists in the two inner rows, but it still remains here and there in the two outer rows. The tangential arrangement may still be evident throughout, but it is frequently lost in the two inner rows. All the cells are considerably elongated tangentially and may reach a length of 35μ. But although the cells have lengthened tangentially, they are not yet crushed. The small air spaces at the corners of the cells have largely disappeared, but large irregular spaces may be found among the cells of the two inner layers. These large "schizogenous" air spaces appear to have originated as a result of tissue distortion and cell displacement. This is of course brought about by the rapid enlargement of the young fruit. The cell wall has become much thicker, especially at the cell corners. The nucleus and cytoplasm are identified with difficulty, especially in cells which are filled with a heavy deposit, and the cells appear to be comparatively inactive. For a similar stage in the varieties studied by Tetley (4, p. 158), she reports that the cell division continues in these sub-epidermal layers. But very careful examination of the McIntosh material failed to reveal any indication of cell division in the hypodermis at this stage.

By the middle of July (Fig. 8) no new radical change has taken place, but all developments outlined for the third week in June are slightly more advanced. Owing, perhaps, to the heavy deposit in the cells, it is practically impossible to identify the protoplasmic cell contents, but the cell outlines may still be recognized everywhere. The changes in cell arrangement and cell shape have extended to the two outer layers just below the epidermis. The cells of these two outer layers are closely packed and without air spaces,

TABLE II

Season and illustrations	Hairs	Cuticle	The epidermis	The hypodermis
Middle of May	Each hair is living and continuous with and part of the epidermal cell from which it has developed. Collectively the hairs form a dense tangled mat with enclosed air spaces, thus providing a very efficient protective layer.	The cuticle is present but is so thin that it may be seen in a tangential section only.	Composed of small, active, thin-walled, isodiametric cells, that are dividing rapidly by radial walls.	Two layers of cells just under epidermis. Cells active and dividing by radial walls. Cell walls thicker than in either epidermis or cortical parenchyma. Cell shape and size similar to that of the cortical parenchyma.
Full bloom	The hairs are now slightly separated by the multiplication of the other epidermal cells. The cuticle is spreading over the side walls of the cell base within the epidermal layer. The cell contents are inconspicuous and as a living cell the hair is becoming inactive.	Continuous and about 1.4μ thick, but not uniform. It is thicker at the bases of the hairs and extends into the hair base and down the side walls within the epidermal layer.	Its cells are now elongated radially to twice their tangential width. They are still dividing and are still thin-walled and full of protoplasm.	Each cell has divided once tangentially; the layer is now four cells thick. Walls have thickened. Cell contents are dense. Nucleus and cytoplasm still evident. Small air spaces still observed at the corners of some cells.
Middle of June	The base is filled in with cuticle and the hairs are now dead. The distal portion is starting to drop off and thus this tissue is no longer effective as a protective layer.	About 5μ thick. It completely fills the hair base and is continuous except over the stomata or young lenticels where it shades off to nothing.	The cells are slightly broader, but the radial measurement is still greater than the tangential. The nucleus is evident, but the cytoplasm is becoming vacuolated. Cell division was not observed. That is the cells are still living but inactive.	Cells of the two inner layers displaced and tangentially elongated. Cell wall continuing to thicken. Cell contents so dense that nucleus can be identified in only a few cells. No indication of cell division. Small air spaces have disappeared from the outer layer; large air spaces are common throughout inner layers.
Middle of July	The hairs are now gone except in and around the depressions at the calyx and stalk ends.	About 11μ thick. It is now fairly uniform in thickness.	The cells have broadened and flattened so that the tangential measurement is now greater than the radial. It is difficult to distinguish protoplasmic contents. There is no trace of cell division, but accommodation to increase in size of young fruit is attained by the cells lengthening tangentially.	Changes in cell arrangement and cell shape have extended to outer layers. Protoplasmic contents indistinguishable. Cells of outer layer closely packed; air spaces throughout inner layers large and numerous. Heavy deposit present in most of cells. Cell walls very thick, especially at corners.

TABLE II—*Concluded*

Season and illustrations	Hairs	Cuticle	The epidermis	The hypodermis
Middle of August	Hairs gone.	About 16μ thick. On its inner surface it is starting to extend between the epidermal cells.	Its cells are starting to separate and the spaces between them are being filled by cuticle. The cell contents are disorganizing.	Cells and air spaces becoming tangentially elongated and radially flattened. Very heavy deposit in practically all cells. All changes in shape, etc., appear to be the result of pressure and stretching. Only vital activity evident is thickening of wall.
Harvest	Hairs gone.	Average thickness about 23μ, but thickness varies from 13 to 25μ. Extends between epidermal cells to hypodermis and even under epidermal cells.	Most of the cells are either flattened or crushed. Many of them are completely separated from adjacent cells by the cuticle, and some are even surrounded by this cuticular invasion. The cell contents have completely disintegrated.	All cells flattened and many crushed. At places exact cellular structure indistinguishable. Walls very thick and frequently laminated. Large air spaces practically eliminated. Most cells filled with dense deposit. No evidence of nucleus or cytoplasm. Layer as a whole very compact. Cells appear to be dead.

but large angular air spaces are evident everywhere throughout the two inner layers. There is still no indication of cell division and the cell walls have continued to thicken. Again it is at the corners that the thickening is most evident. In some of the cells the radial measurement has been reduced, apparently as the result of pressure.

By the middle of August (Fig. 9) the hypodermis starts to assume its permanent appearance. All regular arrangement is gone. All cells are elongated tangentially and flattened radially. Here and there a cell has been crushed. The air spaces are also tangentially elongated. The cell walls have become so thick that in cross section the hypodermis stands out sharply from the other tissues. Nearly all the cells are filled with a very heavy granular deposit and protoplasmic structures are quite indistinguishable. Certainly no cell division is occurring in the hypodermis at this stage and there are no gross morphological changes which could be attributed to the activity of individual cells. The whole hypodermal structure at this time appears to be completely under the influence of stresses and strains due to the enlargement of the fruit. The majority of the cells however are not crushed and they must be living to a certain degree, for the cell walls continue to thicken almost up to the time of harvest. A new feature at this stage is that here and there some of the outer cortical cells become involved in the crushing process and are incorporated as part of the hypodermal protective layer.

During the month of September the hypodermis reaches its final stage (Fig. 10). The cells have become so flattened and crushed, and the walls so thickened, that it is often impossible to identify the limits of an individual cell with any degree of accuracy. The air spaces are so crushed that it is difficult to tell whether certain indefinite tangential slits are crushed cells or crushed air spaces. At places the cell wall is very thick and laminated. The laminae always run tangentially. Again it is impossible to decide whether such a wall is a single, thick, laminated wall or two walls pressed together. No protoplasmic contents are observable and all the cells are filled with a dense deposit. Thus at this stage the hypodermis is a dense mass of closely packed, thick-walled, tangentially elongated, radially flattened or crushed cells. Probably most of these cells are either vitally inactive or dead. The thickness of this mature hypodermis varies from 50 to 100μ.

To make this information regarding the structure and development of the protective layers more readily accessible, it has been summarized in tabular form. (See Table II.)

Acknowledgments

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A PRELIMINARY ACCOUNT OF THE UNIONIDAE OF ONTARIO[1]

BY A. LA ROCQUE[2] AND J. OUGHTON[3]

Abstract

This study is based on an examination of museum specimens and records in the literature. The Unionidae of Ontario comprise 45 species and 18 varieties. Their distribution in the various drainages is shown in a table and the fauna of each area is discussed. The doubtful species and three erroneous records are noted.

Introduction

As an introduction to studies of the distribution of the Unionidae of Ontario, lists that have been published for the Toronto, Hamilton and Ottawa areas and data from more comprehensive works have been brought together, and the specimens on which the lists were based have been examined wherever possible. The taxonomy has been brought up to date (under the direction of Dr. Henry Vander Schalie) and an attempt has been made to indicate briefly the known distribution. Although a large proportion of the shells collected in Ontario have been examined, some species still remain in the "doubtful" list.

The freshwater mussels and clams of Ontario can be separated into two families, the Unionidae (pearly mussels) and Sphaeriidae (fingernail clams). They may be briefly described thus:

Family Unionidae. Length, 1 to 8 in. Shape, varied; some species almost circular, others oval, pendant-like or elongate; some have grooves or knobs on the surface. Color, externally usually brown, green, straw or yellow; sometimes with clearly marked radiating lines; internally white, bluish, silver or rose; often with iridescent hues.

Family Sphaeriidae. Length less than 1 in. Shape, oval or oblong, chiefly, with no conspicuous ornaments. Color, externally straw or light brown; internally white or bluish white.

This account deals with the Unionidae only.

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Contribution from the Royal Ontario Museum of Zoology, Toronto, and the National Museum of Canada, Ottawa, Canada.

[2] *Museum Assistant, Geological Survey, Ottawa.*

[3] *Research Associate, Royal Ontario Museum of Zoology, Toronto.*

Sources of Material

The specimens which have been examined are in the following museums:

1. University Museums (including the Bryant Walker Collection, University of Michigan).
2. The National Museum of Canada.
3. Royal Ontario Museum of Zoology.

These, together with the literature listed below, form the actual basis for this list.

The collection at Ottawa has been partly determined by Simpson and partly by Bryant Walker. Representatives of species in the Toronto collection have been examined by Dr. Vander Schalie.

These collections contain moderately extensive series of shells from the settled parts of Ontario but only a sparse representation from that vast area of the province that is west and north of the Georgian Bay drainage.

We are unable to divide certain genera represented in Ontario into well-marked species. Such "difficult" groups are *Anodonta* and *Lampsilis*. We have not been able to consider their distribution in detail. Likewise *Obovaria* and *Pleurobema* are "difficult" but of less importance since they are not common mussels throughout the province.

The size and shape of the shell in the Unionidae have been shown by Ortmann, Grier and others to be correlated with environmental differences. For example, the shell of a mussel living in a large lake may be dwarfed when compared with that of the same species dwelling in a large slow river. Specimens collected by us in the Niagara River about a mile or two upstream from the Falls, where the current is moderately fast, show similar dwarfing to that induced by lake conditions. We have listed several but not all of these "environmental varieties".

It should be noted that *Amblema costata* (Raf.) and *Amblema plicata* (Say) are herein noted separately, although it seems to us that they are not specifically distinguishable.

We concur in the opinion of Latchford who was the first to point out that *Lampsilis borealis* (A. F. Gray) (7) is no more than a variety of *Lampsilis radiata*, and have treated it accordingly herein. It is interesting to note that the original figures of this "species" were drawn by Justice Latchford and that the initials A. G. on the plate refer to Mr. A. Grignard, the lithographer, and not to A. F. Gray who drew up the description.

A Comparison of Distribution in the Various Ontario Drainages

For this purpose, we have included only 40 species and 14 varieties, having eliminated certain closely related species. The species and varieties of *Anodonta* have been considered as but one species since we feel that many of the species recorded are not valid.

I. *Hudson and James Bay Drainage*

The Hudson and James Bay drainage has a moderately large Unionid fauna but most of the species have been recorded only for Manitoba. This fauna seems to extend only slightly into Ontario. *Lasmigona complanata* may be taken as an example. In Ontario this species is definitely known only from the extreme western portion—Lake of the Woods (Dawson (4)), and Winnipeg River (Latchford (11), Mozley (13)). In Ontario waters of the Hudson and James Bay drainage in general there is apparently a very small mussel fauna, namely eight species and two varieties.

II. *Lake Superior and Drainage*

There are records for only eight species and two varieties—a list equal to that of the above drainage.

III. *Lake Huron and Drainage*

There are 24 species and eight varieties definitely known from this section. It is curious to note that, while *Elliptio complanatus* (Dillw.) is generally common in the Lake Huron drainage, it is apparently very rare in one part, Lake Simcoe. Dr. E. M. Walker, who has for a long time been acquainted with this area, has never found it in the lake. But Rawson (17) has found specimens in the mouths of creeks draining into Lake Simcoe.

IV. *Lake St. Clair, Detroit River, Lake Erie and their Drainages*

These, the most southern waters of the province, contain the greatest variety of mussel fauna, having 34, 26 and 34 species, respectively. Seven species on our list are entirely confined to these drainage areas. *Elliptio complanatus* (Dillwyn) is either absent or very rare here. Ortmann in his Monograph (15, p. 110) says concerning this species: "It has never been found in Lake Erie . . . ."

V. *Niagara River and Tributaries*

The fauna comprises 26 species and four varieties.

VI. *Lake Ontario and Drainage*

We have records for 17 species and four varieties. Two of these species, namely *Leptodea fragilis* and *Proptera alata* are very rare in Lake Ontario. They have been found only in the western end of the lake. It is possible that these two species have migrated there by way of the Welland Canal.

VII. *St. Lawrence (in Ontario) and Ottawa Rivers and Tributaries*

In this drainage section, there are 18 species and five varieties known. Part of this fauna shows affinities with that of the Atlantic slope drainage. For example, *Alasmidonta undulata* is confined, in Ontario, to this drainage section. It is found in the Ottawa River even into the headwater creeks in Algonquin Park but has not been reported from adjacent drainages.

The genus *Lampsilis* exhibits considerable variation in this section. The most "typical" specimens of *L. radiata* have been collected in the Ottawa River, although it has been found in other waters of Ontario.

Summary

1. The pearly freshwater mussel fauna of Ontario comprises 45 species and 18 varieties and of these the greatest number are to be found in the southernmost waters.

2. Seven species are confined in Ontario to Lake Erie, Lake St. Clair, Detroit River, and their drainages.

3. Our present knowledge of the distribution of these mussels, as shown briefly by the list, indicates that the fauna is probably composed of different elements having different affinities and origins. During post-glacial time, when Ontario waters were connected with the Mississippi drainage and with the Atlantic drainage, elements of the Ontario naiad fauna probably migrated from the south and from the east.

Doubtful Species

Anodonta imbecillis Say

This species is recorded from the Michigan side of Lake Erie but as yet we have seen no authentic specimens from Ontario.

Anodonta pepiniana Lea

The status of this species is still in doubt. It may be only a stunted form of *Anodonta grandis*. It has been reported from Lake Superior, from "upper and middle St. Lawrence drainage."

Dysnomia sulcata delicata (Simpson)

This variety is known to occur in the Detroit River but as yet there are no definite records from the Ontario side.

Fusconaia subrotunda (Lea)

The species has been recorded for the Detroit River but so far we have seen no specimens from Ontario. One specimen (NMC 2417) from the Grand River, originally recorded as this species, is *Pleurobema cordatum coccineum*.

Fusconaia undata (Barnes)

This species also has been recorded for the Detroit River but so far we have seen no authentic specimens from Ontario. Two specimens from Port Dover, Lake Erie (NMC 2457) were identified as this species by Simpson, but Walker, who examined them later, decided that one of them was "*Quadrula flava*" and labelled the other "may be *Quadrula undata* Bar. but very doubtful, rather *flava*". Dr. Henry Vander Schalie, to whom this lot was submitted, assures us that both specimens should be referred to *flava parvula*.

Lampsilis ochracea (Say)

All Ontario records appear doubtful.

Leptodea leptodon Raf.

Is recorded for Detroit River. We have seen no authentic Ontario specimens.

Quadrula pustulosa schoolcraftensis (Lea)

Recorded (as *pustulosa prasina*) for Lake Erie by Goodrich and Vander Schalie. We have seen no authentic Ontario specimens.

Simpsoniconcha ambigua (Say)

The species is known to occur in Lake Erie and the Detroit River but as yet there are no definite records from the Ontario side.

FAMILY MARGARITANIDAE:

Margaritana margaritifera (L.)

Recorded for the St. Lawrence River in Quebec but not known for Ontario.

Erroneous Records

The following species have been recorded for Ontario in error:

Actinonaias ellipsiformis (Conrad)

Simpson lists this species for "Southern Canada" and it is recorded by Robertson for the Toronto region, the record being based on specimens in the Brodie collection. This collection is now in the Royal Ontario Museum of Zoology and contains no specimens of this species; neither are there any specimens in the National Museum. Moreover, Dr. Vander Schalie is convinced that this is an error and suspects that specimens of *Micromya iris* were mistaken for this species, which is readily done by one not familiar with the group.

Elliptio crassidens (Lamarck)

Nicholson (1872) lists this species for Toronto Bay, and Robertson (Nat. Hist. Toronto Region, 1913, p. 292) repeats the Nicholson record, but with a question mark. We have never seen specimens of *E. crassidens* from Toronto Bay or from any other part of Ontario. It seems probable, therefore, that the original record was based upon a misidentification.

Pleurobema clava (Lamarck)

The basis for this record is the citation of Call (Trans. Acad. Sci. St. Louis, Vol. 7, p. 9, 1895) who says in part: ". . . . it ranges, as specimens at hand prove, from western New York to Ottawa river, Canada, thence west to Illinois and south to Middle Alabama . . . ." We believe the Ottawa River record to be erroneous, since the species has never been found there to our knowledge although local collectors have been fairly active both before and since the publication of Call's record. So far we have seen no authentic specimens from Ontario.

TABLE I

DISTRIBUTION OF SPECIES

Species	Hudson Bay Drainage	Lake Superior	Lake Superior Drainage	Lake Huron	Lake Huron Drainage	Lake St. Clair	Lake St. Clair Drainage	Detroit River	Lake Erie	Lake Erie Drainage	Niagara River and Trib.	Lake Ontario	Lake Ontario Drainage	St. Lawrence and Trib.	Ottawa Riv. and Trib.
Actinonaias carinata (Barnes)						M	*		M	*	*	*			
Alasmidonta calceolus (Lea)				*		*	*	*	*	*	*	*			*
Alasmidonta marginata (Say)				*	*	*	*	*	*	*	*	9		*	*
Alasmidonta undulata (Say)														*	*
Amblema costata (Raf.)					*	*	*	*	*	*	*				A
Amblema plicata (Say)				*		*		M	*		*				A
Anodonta cataracta Say															
Anodonta grandis Say															
Anodonta grandis benedictensis Lea															
Anodonta grandis footiana (Lea)															
Anodonta grandis gigantea (Lea)															
Anodonta implicata Say															
Anodonta kennicottii Lea															
Anodonta marginata Say															
Anodontoides ferussacianus (Lea)				*	*		*		*	*		*	15	*	*
Anodontoides ferussacianus buchanensis (Lea)				*		*			*						
Anodontoides ferussacianus subcylindraceus (Lea)			*	*	*	*	*	*	*	*	*	*	*	*	*
Carunculina parva (Barnes)			•						*	*					A
Cyclonaias tuberculata (Raf.)							*	M	*						A
Dysnomia rangiana (Lea)						*		*	*						A
Dysnomia triquetra (Raf.)				*		*	*	*	*	*					A
Elliptio complanatus (Dillwyn)	*	*	20	*	*	A?	A?		*?	*?	*	*	*	*	*
Elliptio dilatatus Raf.				*	*		*	*		*	*	*	*?	*	*
Elliptio dilatatus sterkii Grier				*		*			*		*		*?		A
Fusconaia flava (Raf.)					5?		*			*	*				A
Fusconaia flava parvula Grier				*		*			*						A
Lampsilis cariosa (Say)													*		*
Lampsilis fasciola (Raf.)				*		*	*	*	*	*	*				A
Lampsilis radiata (Gmelin)	1		*	*	*		*			*?		*		*	*
Lampsilis radiata borealis (A. F. Gray)	*?		*		*?								*?		*
Lampsilis siliquoidea (Barnes)	*	*		*	*	*	*	*	*	*	*	*		*	*
Lampsilis siliquoidea rosacea (DeKay)	*	15	15	*	*	*			*		*	*	15	*	
Lampsilis superiorensis (Marsh)	*	M	*		*										
Lampsilis ventricosa (Barnes)	*	*?		*	*	*	*	*	*	*	*		*?	*	*
Lampsilis ventricosa canadensis (Lea)	M			*		*			*			*?		*?	*?
Lasmigona complanata (Barnes)	*								*?						A
Lasmigona complanata katherinae (Lea)		*?		*											A
Lasmigona compressa (Lea)	*		M		*	*	*	*	*	*	*	*?	*	*	*
Lasmigona costata (Raf.)			15		*	*	*	*		*	*		*		*
Lasmigona costata eriganensis Grier						*			*		*				A
Leptodea fragilis (Raf.)						*	*	*	*	*	*	*			*
Leptodea fragilis lacustris F. C. Baker						*			*		*?	*			A
Ligumia nasuta (Say)				*		*		*	*	*	*	*	*		A
Ligumia recta (Lam.)				*	*	*		*	*	*	*	*		*	*
Ligumia recta latissima (Raf.)										*			*		*
Micromya fabalis (Lea)							*	*	*						A

The species have been recorded for Ontario. The genus is generally distributed in all drainages.

TABLE I—*Concluded*

DISTRIBUTION OF SPECIES—*Concluded*

Species	Hudson Bay Drainage	Lake Superior	Lake Superior Drainage	Lake Huron	Lake Huron Drainage	Lake St. Clair	Lake St. Clair Drainage	Detroit River	Lake Erie	Lake Erie Drainage	Niagara River and Trib.	Lake Ontario	Lake Ontario Drainage	St. Lawrence and Trib.	Ottawa Riv. and Trib.
Micromya iris (Lea)					*	*	*		*	*	*	*	M		
Micromya iris novi-eboraci (Lea)					*	*	*	*	*	*	*			*	
Obliquaria reflexa (Raf.)							*		*	*					A
Obovaria leibii (Lea)						*	20?	*	*	*					A
Obovaria olivaria (Raf.)					*	*	*	*	*	20	*			*	*
Pleurobema cordatum coccineum					M	*	*	*	*	*	*		20?		A
Pleurobema cordatum pauperculum						*			*		19?				A
Proptera alata (Say)	*?			*		*	*	*	*	*	*	*			*
Proptera alata megaptera (Raf.)				*?			*		*?	*	*				*
Ptychobranchus fasciolare (Raf.)						*	*	*	*	*	*				A
Quadrula pustulosa (Lea)							*		*	*	*				A
Quadrula quadrula (Raf.)							*		*	*	*				A
Strophitus rugosus (Swain.)				*	*	*?	*	M	*	*	*	*?	M	*	*
Truncilla donaciformis (Lea)						*			*	*	*?				A
Truncilla truncata (Raf.)						*	*		*	*					A
TOTALS															
Species	8	4	5	16	16	23	27	23	30	29	24	13	8	12	17
Species (? Record doubtful)	1	1	0	0	1	1	1	0	2	2	1	2	3	0	0
Varieties	2	1	3	7	4	11	4	2	10	4	6	3	3	3	4
Varieties (? Record doubtful)	1	1	0	1	1	0	0	0	1	0	2	1	2	1	1

**Specimens in National Museum of Canada or Royal Ontario Museum of Zoology.*
M—*In University Museums, University of Michigan.*
1, etc.—*Record in literature listed.*
?—*Doubtful record.*
A—*Absent.*

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SOME OBSERVATIONS ON THE OVERWINTERING OF CERTAIN HELMINTH PARASITES OF SHEEP IN CANADA[1]

By Henry J. Griffiths[2]

Abstract

The helminths, *Meniezia expansa*, *Ostertagia circumcincta*, *Nematodirus filicollis* and *Trichostrongylus colubriformis* demonstrated the ability to survive a normal Canadian winter and to cause infection of sheep when ingested during the following spring. Temperatures during the four-month period ranged from −15° F. to 52.5° F. It is concluded that pastures cannot be considered free from these helminths after exposure to such temperatures.

Introduction

While it is an accepted principle of control of helminth parasites in domestic animals that prevention of infection is greatly preferable to destruction of the helminth after it has gained access to the host, it is true that most reliance has so far been placed on anthelmintic medication. This is unfortunate for many reasons, among the most important of which are that few anthelmintics are efficient and all are more or less toxic, and very often much damage has been done by the young parasite before it becomes susceptible to the action of the drug.

This state of affairs has arisen largely because of our lack of knowledge of the bionomics of the helminths and our consequent inability to apply rational, scientific methods of control. At the present time, however, with the limited knowledge available, much can be done by general sanitation, light stocking, pasture rotation, bare lots, and so on, in reducing infestations.

The importance of a helminthic infestation almost always depends upon the number of worms present, and, as no worm can multiply entirely inside the body, the extent of the infection depends ultimately on the number of eggs or larvae on the pasture. It is accordingly essential to have an adequate knowledge of the conditions under which these eggs and larvae will survive in the free state. The following observations on the effects of a Canadian winter on some of these parasites, are presented as helping to throw some light on the free life of these animals.

Results were obtained incidentally, while attempting some pure-strain infestations of sheep with a single helminth. Records of the previous season's experiments on these plots, and the biological data revealed, seemed of sufficient significance to be recorded.

Experimental

Plots

The plots utilized in these trials were small, averaging about 350 sq. yd. each. They were well fenced, and in the early part of the summer yielded fair pasture, comprising a mixture of the following common grasses: white

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[2] *Research Assistant.*

Dutch clover, red top, timothy, Kentucky bluegrass, couch grass and cocksfoot. After a short period of pasturing, supplemental feeds were supplied owing to the exceedingly heavy concentration of sheep on so small an area. Experimental plots were separated from each other by a plot which was not in use. This precaution avoided danger of fecal contamination.

Stock

The lambs employed in this work were from the experimental flock of this Institute. They were born and raised under as helminth-free conditions as practicable, being permitted to suckle their mothers only at certain periods of the day, following the washing of the ewe's udder. Under these conditions lambs have been raised free of bursate nematodes although, in most cases, coccidiosis and an infestation of *Strongyloides papillosus* were not eliminated.

Lambs raised under these conditions have not shown the normal growth and weight that would be observed with lambs running with their mothers at pasture during the summer. However, the lambs have been in good health and have not shown any deficiency symptoms or other physiological disorders.

Fecal Tests and Post-mortem Examinations

Feces were taken, when necessary, directly from the rectum of the individual. The usual centrifugal methods were employed for diagnostic purposes. Post-mortem examinations were carried out in the usual routine by sedimentation of the contents of the intestine; the helminths obtained were preserved in 70% alcohol.

Observations and Results

Plot I. During the summer of 1935, this pen pastured a ewe and a yearling which were on salt lick trials. Throughout the summer fecal tests revealed the sheep to be harboring at least two species of strongyles, and on one occasion tapeworm ova were recorded. These sheep were removed from pasture early in October, 1935. On May 27, 1936, two lambs were put out to pasture in this pen. One lamb was fed a culture of *Haemonchus contortus* larvae, the other being used as a control. Previous to use on this work the lambs were tested and found positive for coccidia and *Strongyloides papillosus* only. On June 6, fecal examination showed the presence of *S. papillosus* and coccidia. On June 30, the experimentally infected lamb showed ova of two species of strongyles and coccidia, the control lamb yielding ova of two species of strongyles, of *S. papillosus* and of a capillarid, coccidia oöcysts also being present. (At this date, June 30, the feces of five control lambs were examined and found to yield only ova of *S. papillosus* and oöcysts of coccidia.) On July 22, fecal tests were made of the two experimental lambs, and analysis revealed the ova of *Nematodirus* sp., strongyles (probably *H. contortus*), and coccidia oöcysts. This test was repeated on July 23 with

identical recordings. This infestation increased and post-mortem results from these lambs were as follows:—

Lamb experimentally infected with *H. contortus:* Killed August 28, 1936	Control lamb, killed July 30, 1936
Ostertagia circumcincta	*O. circumcincta*
Nematodirus filicollis	*N. filicollis*
Haemonchus contortus	*Trichostrongylus colubriformis*
Moniezia expansa	*M. expansa*

Plot II. Throughout the summer of 1935 this plot pastured a ewe, a yearling and a lamb on salt lick experiments similar to those in Plot I. Numerous fecal examinations throughout the summer showed these individuals to be harboring a heavy infestation of helminths, tapeworm ova and four different species of nematode ova being observed. On September 30, 1935, the lamb died, post-mortem examination yielding *Trichostrongylus* sp., *Monodontus trigonocephalus*, and *Chabertia ovina*. During the early part of October, 1935, the ewe and yearling were removed from pasture to winter quarters.

On May 27, 1936, two lambs were pastured in this pen, one animal being fed a pure culture of infective larvae of *C. ovina*, the other being kept as a control. The lamb drenched with the infective larvae died during the last week in June. Unfortunately it was impossible to make an autopsy. Fecal examination of these two lambs on June 11, 1936, revealed the presence of coccidia oöcysts only. On June 30, the control lamb yielded ova of one species of strongyle and of *S. papillosus* on fecal test. (Five control lambs were tested and found to yield only *S. papillosus* ova and coccidia.)

On July 22, 1936, the remaining lamb, on fecal examination, showed heavy infestation of *Moniezia* sp., and ova of a species of strongyle. On July 28, 1936, this lamb was slaughtered, an autopsy yielding the following helminths: *Ostertagia circumcincta*, *Nematodirus filicollis*, *Trichostrongylus colubriformis*, *Moniezia expansa*, *Strongyloides papillosus*.

Plot III. This plot also was utilized for salt lick trials during the summer of 1935, pasturing a ewe, a yearling and a lamb. Numerous fecal examinations of these individuals throughout the summer showed them to be harboring three species of strongyles and tapeworms. These sheep were removed from pasture early in October, 1935.

Two lambs were pastured in this pen on May 27, 1936. Fecal tests on these animals on June 11, 1936, showed an infestation of coccidia only. On June 30, 1936, the examination revealed ova of a species of strongyle and of *S. papillosus* and coccidia oöcysts. (Five controls examined on the same date were found to be carrying *Strongyloides* ova and coccidia only.) Fecal examination of these lambs on July 22, 1936, showed them to be harboring

two species of strongyles, *S. papillosus* and coccidia. Post-mortem examination of these lambs yielded the following helminths:

Killed July 22, 1936. *O. circumcincta, N. filicollis, S. papillosus.*

Killed July 24, 1936. *O. circumcincta, N. filicollis, M. expansa.*

Controls

Of the 11 lambs available for experimental purposes, five were kept as controls throughout the summer of 1936, to be used later for other purposes. These five lambs and those selected for use on the experimental plots were raised under identical conditions. They had every opportunity of picking up an infestation of helminths should the technique employed in raising helminth-free lambs have proved unsatisfactory. All the lambs were recorded as carrying *S. papillosus* and a coccidial infection at some time or other and, consequently, these two parasites were disregarded throughout the investigation.

Until June 29, 1936, these five control lambs were kept indoors in concrete pens. On that date the lambs were put out in small paddocks which were known not to have been pastured by sheep for many years. Fecal tests were made of these control lambs at irregular intervals, and at no time were any ova observed except *S. papillosus*. Coccidia oöcysts were observed on most occasions.

Resistance to Winter Temperatures

Certain helminths mentioned in this paper have been shown to possess the ability to resist the low temperatures of a Canadian winter. These helminths not only have the power to withstand comparatively low temperatures, but also appear to possess the ability to withstand freezing and thawing to a considerable extent.

Whether this resistance mechanism is functional in the ovum or the infective larvae is still debatable, but from the observations of other investigators it is probable that infective larvae would withstand the rigors of winter to a far greater extent than would the ova.

The maximum and minimum temperatures for a four-month period of the winter of 1935–36, are charted in Fig. 1. These temperatures were obtained through the courtesy of the Department of Horticulture, Macdonald College, McGill University.

It will readily be noted from the graphs that some sharp rises and falls in temperature were recorded during the four-month period. The maximum and minimum temperatures, as indicated in the graphs, for December, 1935, and January and February, 1936, will be observed to be mostly below 32° F. At several periods during these months the mercury dropped to zero or well below, the minimum temperature recording well below zero for several days at a time in some instances. In the first few days of March the temperature dropped to −11° F. This was followed by milder weather, though at no period during the month did the minimum recorded temperature rise much above freezing point.

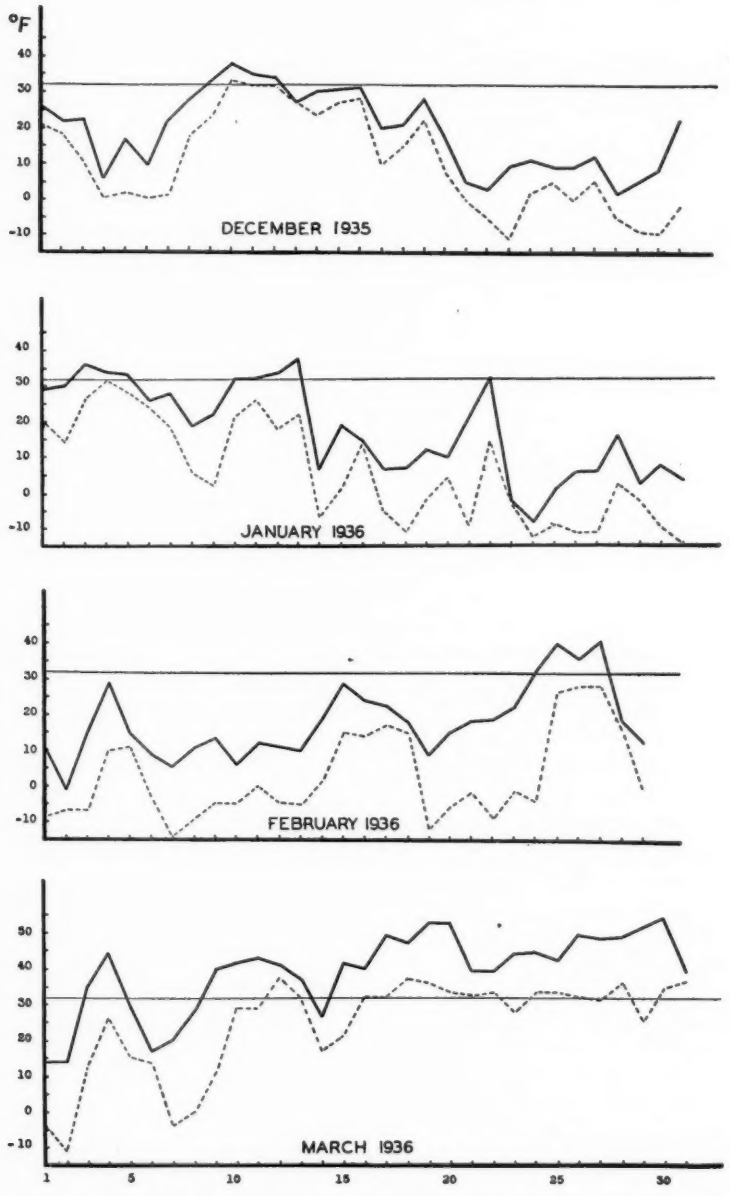

FIG. 1. *Graphs showing maximum and minimum temperatures for a four-month period in the winter of 1935–36. Maximum temperatures indicated by continuous line, minimum by dotted line.*

December, January and February were slightly colder than the average for the island of Montreal, although March was warmer (Table I). The precipitation for January and February was normal—both rain and snow—but in December both rain and snow were considerably less than usual, and in March rain was above average while snowfall was below average. It seems therefore that the winter of 1935 in this district was slightly more severe than normal and that the worms which survived this winter might be expected to survive any normal winter in the Montreal area.

TABLE I

TEMPERATURE AND PRECIPITATION

—	December		January		February		March	
	Average	1935	Average	1936	Average	1936	Average	1936
Temperature, ° F.								
Maximum	26	19.3	21.0	17.9	22.0	17.9	33.0	39.0
Mean	20	14.8	13.0	11.9	14.0	10.3	26.0	31.55
Minimum	13	10.4	5.0	6.0	7.0	2.72	18.0	24.11
Total precipitation	3.74	1.245	3.75	3.27	3.17	3.05	3.52	4.41
Snowfall, in.	23.7	9.75	28.5	28.5	24.4	25.5	24.4	11.25

Average figures taken from Canada Year Book for 1931. (Total precipitation includes snowfall calculated at 10 in. snow = 1 in. rain.)

Discussion

Up to the present, few data have been available on the lethal effect of Canadian winter temperatures on the non-parasitic stages of the common helminth parasites of sheep while on pasture land. It has been possible, from the information available on the bionomics of certain parasites, to predict the improbability of their survival throughout the winter. Undoubtedly the survival of helminths would not depend merely on one factor such as temperature, but on a complex of factors, such as precipitation, period of exposure to a certain temperature, snow-cover, type of herbage, location of pasture and many other factors.

The small number of experimental animals employed may be criticized but the observations recorded were significant, not relative to the numbers employed, but to the fact that the animals used must have been raised without possible chance of bursate nematode infestation. *Strongyloides papillosus* was observed in most cases, and on account of the certainty with which the ovum can be identified, was disregarded throughout the experiment. It has been found practically impossible to raise lambs free of this parasite.

Chance contamination of the lambs exposed to the "pure-line" infection in each pen could possibly have occurred during the culturing and preparation of the infective larvae for drenching. This chance, however, was eliminated by the use of a control lamb in the two pens where pure line infections were attempted.

Conclusion

The following common helminths of sheep demonstrated the ability of being able to survive a normal Canadian winter and to cause an infestation within the sheep when ingested at pasture during the following spring: *Moniezia expansa, Ostertagia circumcincta, Nematodirus filicollis, Trichostrongylus colubriformis.*

These species were able to withstand temperatures ranging from −15° F. to 52.5 ° F. encountered during the four month period.

It is apparent that pastures cannot be considered as free from these helminths after exposure to climatic factors experienced during one normal Canadian winter.